The effect of feeding regime on larval black fly (Diptera: Simuliidae) primary head fan ray number

by

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Abstract

Identification of larval simuliids has always been difficult due to the morphological similarity many species bear to one another. For this reason all characters available have been drawn upon to aid in species identification, including head fan ray number. Even in light of an increasing body of anecdotal reports that head fan ray number is not fixed, it has continued to be used to aid species identification. In the current experiment simuliid larvae were reared under controlled laboratory conditions to last instar in one of three feeding regimes. Out of nine trials, the results of six showed a significant inverse relationship between feeding regime and head fan ray number. In addition to the laboratory experiments, larvae were also collected from the field over the course of the spring and summer, 1994. From these samples significant interspecific and intraspecific variations in head fan ray number were found both spatially and temporally within Algonquin Park.

From these data it is concluded that head fan ray number for the species analysed is a developmentally plastic character, which varies in response to food availability. Furthermore, given the extreme variations in head fan ray number found in some species, I recommend that head fan ray number not be used as an aid to identification unless it can be shown to be a fixed character for the species in question.

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Introduction

Black flies are true flies in the suborder Nematocera. Their family name, Simuliidae, derived from the Latin word *simulium*, means 'a little snub nosed being', which broadly describes their appearance. Larval simuliids are arguably the most successful creatures of the lotic environment and are almost perfectly adapted for their way of life. Fossil records dating back to the Upper Jurassic reveal larvae almost indistinguishable from those studied today (Kalugina 1991). The number of named species of black flies exceeds 1600 with certainly many more yet undescribed (Crosskey pers. comm. 1995).

Many species of black fly are particularly successful both in distribution and numbers. *Simulium malyschevi,* for example, can be found in Alaska, Canada, China, Japan and the USSR (Kim and Merritt 1987). Larval conglomerates of *S. noelleri* may number $1.2x10^{-6}$ per m^2 carpeting the substrate so densely it is no longer visible (Wotton 1987). Black flies have colonised most land masses, and almost every area of those land masses where there is enough fresh flowing water and there are sufficient nutrients for larval development.

However, members of this dipteran family are much more often noted for being pernicious pests than for their evolutionary successes. Their mastery of the terrestrial environment is overshadowed by their considerable numerical representation and attacks by blood-sucking females of many species. The opinion that the general public has of black flies has been reflected in the research carried out by the scientific community, as can be judged by the number of papers that have investigated black flies in relation to their pest status.

Life history

The black fly life cycle, like that of other true flies, is divided into four discrete stages - egg, larva, pupa and adult - in chronological order. The first three stages are primarily aquatic. Typically, black flies are univoltine; however, a few species have eggs which can undergo a period of aestivation under adverse conditions (Crosskey 1990, Rubtsov 1956).

Eggs

Simuliid eggs are very small, rarely greater than 0.5 mm in length. Eggs are difficult or impossible to identify to species (Timm 1987) and coupled with their minor impact in the lotic environment, have been poorly studied. This is unfortunate because not only does the maximal population of a cohort occur in this stage, but possibly also the greatest level of immature stage mortality (Colbo 1987). When laid, eggs are pale white in colour and they gradually turn a dark brown as the embryo develops. They are asymmetrically ovoid in shape, varying from triangular or reniform (kidney shaped) in one aspect to near oval in another (Crosskey 1990, Peterson 1981, Rubtsov 1956).

The survivability of eggs is poorly understood. Colbo and Moorehouse (1974) demonstrated that eggs of *Austrosimulium pestilens* can survive for at least two years. This is a significant time period when compared to the remainder of a black fly's life cycle, which is typically no more than a few weeks.

Larvae

Newly hatched, first instar, larvae are easily recognisable by the presence of an egg-burster on the dorsal side of their head capsule. The egg-burster is retained until the cuticle is shed at the first moult (Davis 1971). Typically characteristic of all larval stages is a pair of labral fans or head fans which are

used to filter the lotic environment (Craig 1974). Black fly larvae have a very distinct body shape which is often likened to a juggler's club, in which the larva's posterior is the fat end (Figure 1).

One of the most conspicuous characters of nearly all larvae is the primary head fans. These are paired structures that arise from the apex of the head fan ray stalks situated on the anterolateral corners of the head (Figs. 1 and 2), and are synonymous with the structures known as labral fans (Craig 1974). In addition to the primary head fans there are also a pair of secondary head fans which lie laterobasally to each primary fan, and a pair of medial fans which lie on the medial side of each head fan stalk (Chance 1970). Each fan is composed of numerous fan rays and, for the primary fan at least, the number of rays constituting the fan varies both interspecifically and intraspecifically (Adler and Kim 1986, Craig 1974). The secondary and medial fans are smaller than the primary head fans, typically bearing approximately one-half and one-quarter of the rays of the primary fan, respectively. The rays themselves for both the secondary and medial fans are also shorter than those of the primary head fan (Chance 1970).

Head fans of first instar larvae, although smaller and bearing fewer primary fan rays, are typically similar in both structure and function to those of later instar larvae. Within the first few moults extra fan rays are recruited to the fan structure. The exact method of recruitment is undocumented, but each fan ray is derived from a single cell (termed a progenitor) each instar (Craig 1974, Craig pers. comm. 1995).

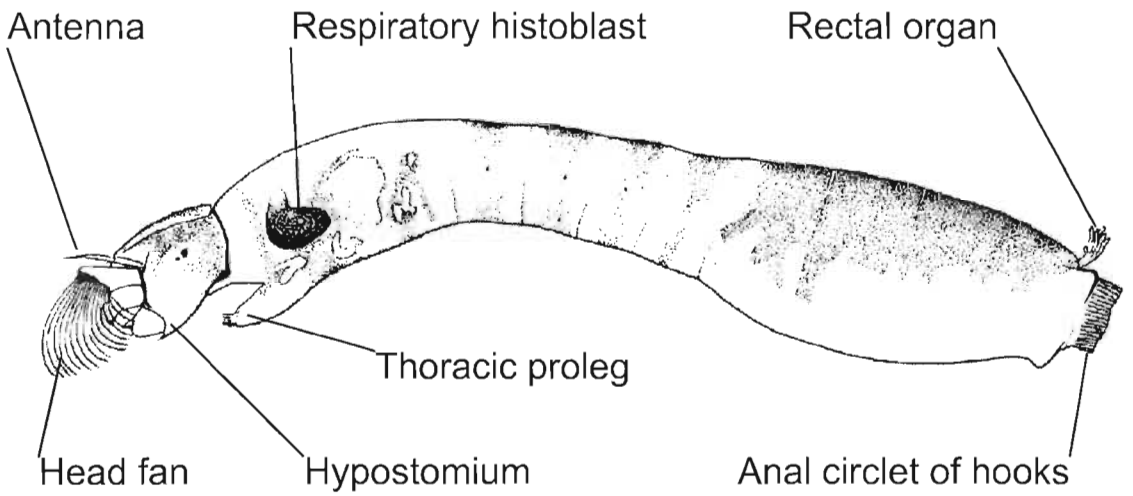

Figure 1. Larval black fly structure (lateral view), modified from Crosskey (1990).

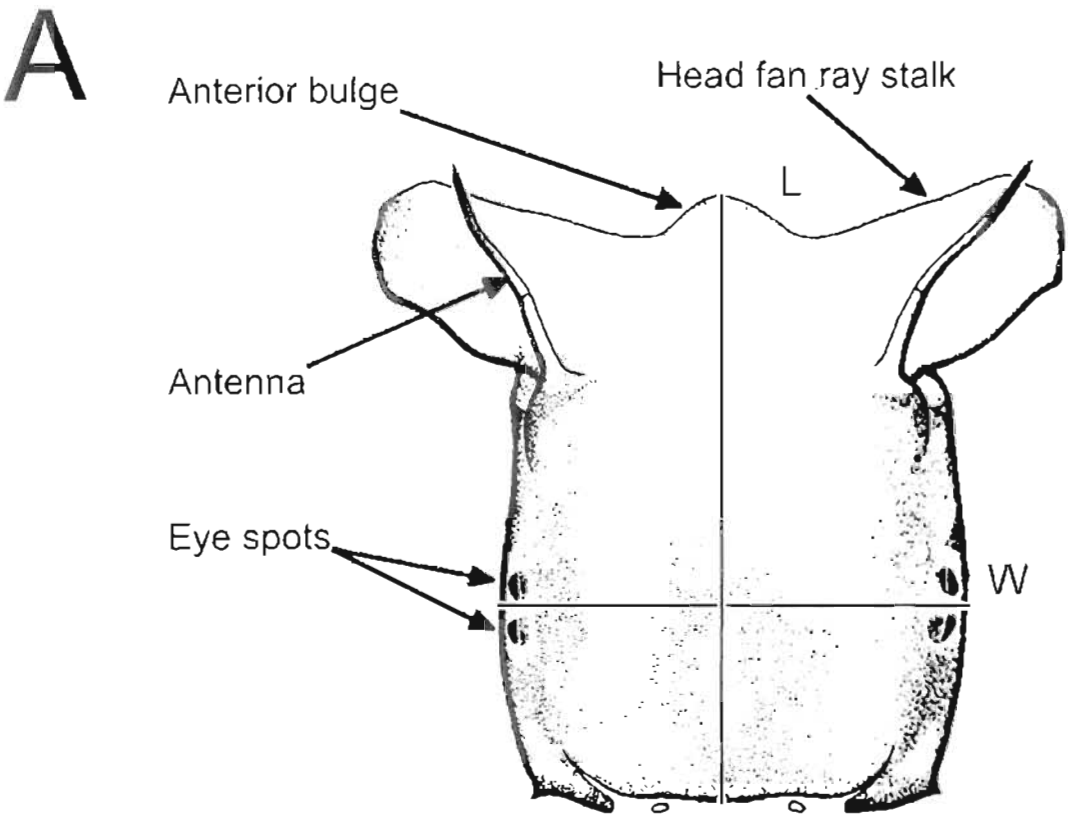

Figure 2. (A) Diagram showing dimensions of the larval heads measured, length (L) and width (W), modified from Currie (1986). Head fan rays omitted for clarity. (B) Photograph of larval head showing head fan rays.

The number of instars through which a larva passes can vary from 4 to 9, depending upon the species (Craig 1975, Yakuba 1960). Typically, larvae pass through a maximum of 6 or 7 instars (Crosskey 1990). Ross and Merritt (1978) have shown that for some species the number of instars through which the larvae pass during development decreases with increased temperature. Excluding an increase in the number of labral fan rays as the larvae moult, different larval instars are morphologically very similar to one other. However, the last larval instar, or pharate pupa, is only externally similar to other instars. Internally, it is a pupa which effects movement of the larval cuticle via apodemes. Pharate pupae are characterised by conspicuous dark histoblasts that show through the larval cuticle just posterior to the head capsule, laterally on either side of the thorax (Crosskey 1990, Hinton 1958).

Simuliid larvae are well adapted to the lotic environment in which they live. In particular, they possess a pair of large silk glands which can secrete copious amounts of silk that they work with their mandibles. The main roles of the silk are to aid in anchoring the larva to the substrate and movement. A larva will secrete a small amount of silk which it works onto the substrate where it sticks. This provides a secure base into which the larva can bury its anal circlet of hooks (Barr 1984) (Fig. 1). Movement is achieved by the larvae reaching out horizontally and working a new pad of silk onto the substrate. Then, gripping the new pad with mandibles and thoracic proleg the larva releases its anal circlet of hooks from the old pad, and looping its body, brings the anal circlet of hooks to the new pad and reattaches itself. By repeating this process larvae can move over any contiguous solid substrate, both up and down stream (Barr 1984, Crosskey 1990).

Pupae

The most important role of the pharate pupa is to spin a secure and protective silk cocoon in which to pupate (Hinton 1958). If not completed correctly, or at all, the probability that the pupa will survive is likely to be very much reduced. This aspect of the simuliid life cycle is poorly understood and is now being studied with the detail and attention it deserves (A. Stuart, Brock University, pers. comm. 1995). The complexity of the cocoon spun varies greatly among species. However, intraspecific uniformity of cocoon construction is remarkably stable and can aid in species identification (Adler and Kim 1986, Crosskey 1990).

Having spun the cocoon the pharate pupa moults and becomes the true pupa (Hinton 1958). The pupa and cocoon are aligned longitudinally with the flow of water, posterior end upstream. This orientation facilitates gaseous exchange by holding the respiratory filaments, situated anteriorly, in the flow of water. The branching pattern and number of respiratory filaments show considerable diversity interspecifically. Consequently they are used extensively for identification, even for pharate pupae, since they can easily be dissected out of the histoblasts (Currie 1986, Peterson 1970).

As with the larval stage the length of the pupal stage is very much temperature dependent, but it is never very long. Typically adult emergence occurs between 3 and 10 days. Rarely does the pupal stage exceed 14 days even in arctic species (Crosskey 1990). Black flies have an extraordinary way of leaving their aquatic cocoon and becoming aerial. As the pupal skin splits open the adult fly becomes enveloped in a bubble of air. The now buoyant fly floats to the water surface and is released to its terrestrial life. Some species, such as

S. damnosum, are able to take immediately to flight (Burton 1966). More typically, however, it is necessary for the black fly to float to a dry surface where it waits for its wings to harden before taking to the air, e.g., *S. arcticum* (Shemanchuk 1987).

Adult

Morphologically, black flies are very distinct from other Diptera. In colour they are typically black or blackish brown, although yellow and orange species are known. They are quite small, 1.2 to 6.0 mm in length with a typical wing length of 1.4 to 6.0 mm. Their overall appearance is dominated by a pronounced thoracic scutum. This gives them a 'humpbacked' appearance and explains why they are sometimes called 'Buffalo-gnats'.

The two sexes can be differentiated by the sexually dimorphic character of the eyes. Males are typically holoptic and females always dichoptic. Males of species where both sexes are dichoptic are noted for their inability or unwillingness to fly (Wood 1978). One of the most characteristic behaviours of adult males is the formation of a mating swarm, which if adhered to in its extreme definition, may be composed of a single male fly (Wenk 1987). The swarm, or lek, minimises male dispersion and improves the chance of meeting and mating with females. Although swarming is a characteristic behaviour of male black flies of most species it is not obligatory for inducing mating. In some species, typically those where the males are dichoptic, e.g. *Cnephia dacotensis*, aerial swarming never occurs and mating occurs on the banks of the water course from which the adults emerged (Crosskey 1990, Edman and Simmons 1986, Wenk 1987).

Males detect females primarily by sight, demonstrated by the fact that males readily chase any object similar in size to a female thrown or swung past a swarm. Black flies are unable to copulate in flight. Thus, paired individuals fall from the swarm and copulate on the ground, a process which takes from 30 to 120 seconds (Crosskey 1990, Wenk 1987).

The most noteworthy aspect of adult black fly life is the blood meal. Only required by females, and then not in all species, the blood meal is often obligatory for the development and maturation of the eggs (Anderson and Shemanchuk 1987, Le Berre 1966). Obligate anautogeny (in which a blood meal is required to complete the female reproductive cycle) is only one of the four basic reproductive strategies. The others are obligate autogeny (where a blood meal is never required or taken to complete the female reproductive cycle), facultative autogeny (where a blood meal is taken by typically autogenous females emerging from nutrient poor larval habitats) and facultative anautogeny (where a blood meal is not taken by typically anautogenous females emerging from nutrient rich larval habitats) (Anderson 1987).

Unlike many other blood feeding insects female black flies do not have piercing sucking mouthparts (Peterson 1981). Instead, they first press their labrum against the skin of the host, bending the labrum back in the process and stretching the skin of the host tight, before cutting the skin open with the mandibles. In response to tasting adenine nucleotides in the blood, the female black fly will commence sucking blood. Sucking is accomplished by co-ordinated action of the cibarial and pharyngeal pumping muscles, and the oral and post pharyngeal constrictor muscles (McIver and Sutcliffe 1986). Blood meals are taken solely by females for egg development, whereas energy for other activities, such as flying, is taken by both female and male flies in the form of a

sugar meal.

Black flies typically feed on sugar solutions. To do so, they extend the labella into the solution, which is drawn up via capillary action to the oral labellar cuticle before being pumped down the food canal by the same processes as described above (McIver and Sutcliffe 1986). The sugar meal was originally thought to be constituted of only nectar and is generally referred to as such (e.g., McIver and Sutcliffe 1986, Crosskey 1990). Although nectar is known to be a source of carbohydrates for many species of black fly, homopteran honeydew has now also been identified as a source of sugar for several species (S. Burgin, Brock University, pers. comm. 1995).

Fecundity of mated females varies considerably among species and with respect to age, size and condition (Davies and Peterson 1956, Mokry 1980, Pascuzzo 1976). Size and condition, and consequently fecundity, of adults is directly related to the size and condition of the larvae at pupation (Colbo and Porter 1979).

The life cycle is completed with the laying of mature fertilised eggs by the females. Eggs are unable to survive desiccation and so for all species they must be laid in water or at least in a damp atmosphere (Crosskey 1990, Timm 1987). Four oviposition techniques have been observed among simuliids: scattering (eggs tapped or dropped onto the water surface), dabbing (eggs laid singly, or in small groups, on solid substrates), stringing (eggs arranged in irregular lines on solid substrates) and layering (eggs arranged in compact flat groups on solid substrates) (Golini and Davies 1986, Crosskey 1990).

There is no parental care given to oviposited eggs other than site selection by the female. Having oviposited one batch of eggs female black flies are capable

of undergoing a further gonotrophic cycle to produce more eggs (Anderson 1987).

Black fly ecology

As previously noted, black flies have successfully colonised all but the most inhospitable areas of the globe. Yet, in those areas where they are not pest species the general public are all but unaware of their very existence. Remarkable among the black flies is the specificity of certain conditions required to complete their life history, such that laboratory colonies are notoriously difficult to establish and maintain (Edman and Simmons 1986). Indeed, in areas where there has been considerable ecological disturbance, for example urban development, black flies unlike many insects are very rare.

Researchers have had considerable success rearing larvae from eggs to last instar larvae, through pupation and even to the adult stage. As Edman and Simmons (1986) note, rearing larvae to adults is not, in itself, an indication of complete success. The optimum conditions of water quality and diet for rearing larvae have not been determined. It is known only that by adhering to standards already in use acceptable rearing results can be obtained.

Adult survival in laboratory colonies is very low, even given highly controlled conditions. Mortality typically reaches 80% a mere two weeks after emergence. There is also the problem of a failure of the flies to mate, even in those species where the formation of a swarm is not a component of normal mating behaviour. One procedure that does achieve modest success, but with selected species only, is crowding - whereby a large number of black flies of both sexes are confined to a small volume (Edman and Simmons 1986).

Although the number of female black flies that will blood feed and oviposit under laboratory conditions is often well below 100%, current techniques do achieve adequate results. Given 'body' temperature blood through an artificial membrane black flies seem more than willing to feed. And, while techniques to induce oviposition vary, inducing 70% or more of females to oviposit is common (Tarrant *et al.* 1987).

Larval microdistribution

A brief look in a water course will clearly show that larvae are not attached evenly over every surface. There are always patches of low and high density, and also patches that are strangely devoid of any individuals at all. The delimitation between such patches is often an acute one, almost as though there were an invisible barrier.

At the population level gross distribution patterns seem most likely dictated by water flow. Water flowing at less than 2.5 cms^{-1} will not pass through the rays of a head fan, and filter feeding effectively stops (Lacoursière and Craig 1993). Not until the rate of flow exceeds 10 cms^{-1} is there an appreciable filtration of particles from the water by head fans of larval black flies (Braimah 1987b, Horne *et al.* 1992). Thus, filter feeding species require a site with a fast enough flow of water. What though, are the optimum and maximum rates of flow chosen by black flies?

Maximum suitable rates of flow almost certainly vary from species to species, depending upon such factors as strength of attachment and head fan rigidity. At extremely fast current velocities larvae are likely to be either swept away or suffer from their head fans collapsing, again effectively preventing feeding. However, a field analysis of current velocity flowing past larval

microhabitats has shown larval *S. virgatum* to be living in flow rates of up to 210 cms^{-1} (Eymann 1993).

Trying to determine the optimum water velocity for black fly larvae is difficult and it is likely not a determinant of feeding efficiency. For example, two simuliid species, *Austrosimulium furiosum* and *S. ornatipes* often co-occur. However, under laboratory conditions they have been shown to prefer water velocities of 20 - 30 cms^{-1} and 90 - 130 cms^{-1}, respectively. Most researchers place optimum feeding efficiency for black fly larvae, with respect to water velocity, in the region of 15 - 30 cms^{-1} (Braimah 1987c, Kurtak 1978, Lacoursière and Craig 1993). However, in the field Eymann (1992) studied 12 species of black fly larvae and most were found in flow rates of 40 - 90 cms^{-1}.

The high water velocity preferences of larval black flies may in part be a behavioural adaptation against predation. For the triclad flatworm *Dugesia dorotocephala* at least, water velocity is a significant mediator of predator-prey interactions with black fly larvae. In controlled water velocity preference experiments, larval *S. vittatum* chose significantly higher rates of flow than *D. dorotocephala.* The water velocity chosen by nearly 60% of the black fly larvae was greater than that in which the predatory triclad worms could maintain a normal level of activity (approximately 40 cms) (Hansen *et al.* 1991).

Although many other physical factors may affect a population of black fly larvae, such as temperature, pH and substrate type (Ross and Merritt 1986), few if any are likely to affect the distribution of larvae over a substrate. Biological factors such as larval interactions and food concentration affect microdistribution, and these will be discussed below. However, the relative effects of both aggression and food availability are directly tied to water

velocity.

Aggression in black fly larvae towards other individuals is not consistent and can vary according to food concentration and water velocity (Ciborowski and Craig 1989, Dudley *et al.* 1990, Hart 1987, Hart and Latta 1986). Theoretically, territoriality should not arise unless resources are actually or potentially limiting, and are economically defensible. Successful defence of a territory by an *S. piperi* larva directly increases feeding success (Hart 1986). Hart's (1986) research demonstrated that; (1) aggressive behaviour is nearly always directed towards upstream neighbours, (2) ingestion rates increase significantly for larvae successful in displacing upstream neighbours and (3) by artificially increasing food concentrations, aggression can be significantly reduced.

However, black fly larvae are relatively ineffectual filterers, typically removing less than 1% of the total available filterable material from the water (Hart and Latta 1986, Morine *et al.* 1988). Thus it is unlikely that food concentration alone describes the observed aggressive and territorial behaviour as, in general, upstream neighbours remove an almost insignificant amount of material. More probably, as water passes around upstream larvae it becomes turbulent, slows, and forms small vortices. This disruption to a laminar flow will not only increase drag forces on the larvae downstream but will also reduce the filtering efficiency of the head fans (Ciborowski and Craig 1989, Craig and Galloway 1986, Dudley *et al.* 1990, Hart 1987). In Hart's (1986) experiment, it is likely that the food concentration was increased to an extent where the turbulence created by an upstream neighbour became insignificant in relation to feeding success.

Medical and veterinary importance of black flies

As previously mentioned black flies are viewed as serious pests. Although when considering the clinical effects of the pathogenic nematode, *Onchocerca volvulus,* that some species carry and the economic losses black flies have caused in the farming community, the term pest seems an inadequate description.

Vectors of disease in humans

The only pathogen conclusively known to be transmitted to humans by black flies is the microfilarial nematode *O. volvulus*, which causes onchocerciasis, better known as river blindness. The nematode occurs over a large part of western central Africa, where the black fly vector is predominately the *S. damnosum* complex. The nematode also occurs in several areas of Latin America, where there are eight black fly vectors. The three principal species are *S. ochraceum*, *S. metallicum* and *S. callidum* in order of importance (Takaoka and Suzuki 1986, Crosskey 1990). In these areas river blindness constitutes a serious human pathogen (Philippon 1986).

River blindness is not in itself a terminal disease. The human body is more than able to tolerate the presence of the nematodes. However, symptomatic of infection is blindness, caused by nematode microfilariae that migrate to the cornea of the eye and die. Their death brings upon the opaqueness of the cornea typical of infection. As increasingly large numbers of nematodes die ultimately light is restricted from entering the eye (Crosskey 1990).

In West Africa, life expectancy of blind people is approximately 13 years less than those with normal vision. This translates to 22 years lost production per afflicted person. In regions with the highest infection rates 25 - 50% of adults

may be blind, constituting a considerable loss of human resources and economic drain in the area (Philippon 1986, Crosskey 1990).

Vectors of disease in livestock

Pathogenic diseases carried by black flies that affect livestock fall into two main categories. Cattle onchocerciasis is caused by a number of species of microfilarial nematodes, *Onchocerca spp.*, and leucocytozoonosis in poultry is caused by protozoan blood parasites of the genus *Leucocytozoon* (Cupp 1986).

In Southern England infection rates of *Onchocerca spp.* in cattle may reach 84%. However, the disease produces no clinical effects and is restricted to tissues not eaten by humans. For such reasons it is of low economic importance (Crosskey 1990). *Leucocytozoon spp.* in poultry, however, can be particularly pathogenic to ducks, geese, chickens and turkeys. The disease causes death and reduced egg production in many areas of the world, particularly the United States and is of significant economic importance (Cupp 1986, Crosskey 1990).

Black flies as pests

Black flies are also pests in a direct sense, in that their biting causes irritation and discomfort. In those individuals who suffer allergic reactions to black fly bites, however, the result of even a single bite can be quite serious. In general, however, a black fly bite is quite innocuous.

Black flies can and do occur in vast numbers on some parts of the globe, such as Russia, North America, South America and Australasia, to the extent that some areas become almost uninhabitable for parts of the year when numbers of flies are very high. In such areas the economic impact can be quite high as a result of lost tourism and decreased productivity in out-door jobs

(Fredeen 1973, Fredeen 1986, Crosskey 1990).

The greatest economic impact from black flies in North America comes from attacks against livestock. Domestic animals, unlike humans, are less able to avoid the effects of a mass attack by black flies, resulting in reduced meat and milk production, and even death. Death is caused by blood poisoning from the female black fly's saliva (Anderson and Voskul 1963, Crosskey 1990), by suffocation due to livestock having inhaled large numbers of flies or from anaphylactic shock.

Black fly parasites and predators

Far from being a pest to many species, black flies are important as prey items (Davies 1981, Goncharova 1983) and hosts (Molloy 1986). Crosskey (1990) has surely collated the largest inventory on both the predators and hosts of both the larval and adult stages of black flies. From the literature it soon becomes apparent that there are few examples of either predators or parasites inflicting a serious impact on black fly numbers. However, for some predators, black flies can constitute a significant proportion of dietary intake (Davies 1981, Goncharova 1983, Merritt and Wotton 1988), while for some parasites black flies are an obligatory host (Lacey and Undeen 1986).

The main aim behind the investigation into the predators and parasites of black flies is in the hope of discovering a suitable biological control method. In the case of predators, some fish have been seen to feed heavily upon black fly larvae (Davies 1981, Goncharova 1983). However, the predation by fish on black fly larvae is viewed only as an opportunistic behaviour, there being no evidence to support the existence of a black fly eating specialist. Merritt and Wotton (1988) describe the life history and behaviour of the predatory dipteran

Limnophora riparia, a black fly eating specialist. The larval life history of *L. riparia* coincides spatially and temporally with that of larval *S. noelleri*, its main prey. Although they found that at two sample sites larval numbers of *L. riparia* correlated to larval numbers of *S. noelleri* they do not suggest *L. riparia* is an important predator.

Possibly of greater importance in affecting black fly numbers comes through the effects of parasitism. Both larval and adult black flies are known to be hosts to protozoans, fungi, bacteria, nematodes and viruses. However, as is typically the case with most parasites infection rates among hosts is low, except in some cases where the parasite is non-lethal (Lacey and Undeen 1986, Crosskey 1990).

The use of parasites as a method to control black fly numbers has received intensive research with most effort being placed on the control of larvae. A number of parasites have been isolated, cultured and subsequently, they been demonstrated to infect larvae. However, the cost of biological control still remains prohibitively high and the results are too sporadic for commercial use.

Head fans

There are a number of genera in which the labral fans (Fig. 1 and Fig. 2), so conspicuous in most larval black flies, are either considerably reduced or absent, although a lack of head fans is considered a secondary characteristic (Craig 1974). For example, first instar larvae of *Twinnia spp.* bear only the most rudimentary non-functional head fans, which are subsequently lost at the first moult. *Gymnopais spp.* larvae never bear head fans of any complexity throughout their life history (Craig 1974). However, within the family Simuliidae those genera and species which do not bear head fans are few and for the most

part can be considered atypical.

Each primary head fan is a composite of a number of discrete sickle-shaped fan rays, each approximately 0.3 to 0.5 mm in length. By increasing the pressure of the body fluids within the fan stem the black fly is able to force the fan into its open, abducted, position. When opened, the fan approximates a quadrant of a hemisphere, approximately 200 to 250 degrees across. The fan is closed, adducted, to the resting position by contraction of the cephalic fan retractor muscle. When retracted the primary fans rest neatly against the labrum and mandibles. A cycle of opening and closing the head fans is termed a 'flick' and is sometimes used to estimate feeding rate (Chance 1970, Craig 1977b, Hart 1986).

The primary fan rays are made of hard, flexible cuticle and bear numerous microtrichia down their inner curved surface. The development of the fan rays is quite extraordinary and homologous to the development of the palatal brush in the mosquito larva *Aedes aegypti* (Fry and Craig 1995, Fry and McIver 1990). Immediately after ecdysis of one instar a large apolysial space develops between the labral cuticle and the underlying epidermal cells. Within this space, the pharate labral structures of the following instar form, including the head fans. Each fan ray develops from a single progenitor cell that elongates within the apolysial space. After elongation layers of cuticulin, inner epicuticle and procuticle are deposited sequentially on the outer surface of the cellular extension. With the deposition of the final layers of cuticle there is extensive organelle breakdown within the cell.

As previously mentioned, each palatal brush filament in larval *A. aegypti* develops from a single cell. However, more importantly, each filament-forming

cell will develop a filament. Consequently, the increase in the number of filaments that occurs between successive instars must be due to cellular division at some point during instar development. The point at which this cell division occurs is unknown, and it is not clear whether this system of filament recruitment is analogous to the increase in head fan ray numbers observed in larval simuliids (Fry and Craig 1995, Fry and McIver 1990).

Microtrichia form the finest structure of the filter mechanism of the head fan. Typically, they are small, linearly arranged projections on the inner (upstream) surface of each ray. In many species they are of uniform length interspersed regularly by a single long one e.g., *Prosimulium fuscum*, and *S. venustum* (Chance 1970). Other common patterns include a saw-tooth-like arrangement, e.g., *S. bivittatum* (Craig 1986) or uniformly sized microtrichia, e.g., *S. tahitiense*. *Cnephia dacotensis* has a unique trichiation arrangement, in that it is has two rows of long irregularly spaced microtrichia that form a 'V' shape as they extend from the upstream edge of the head fan ray (Chance 1970, Craig 1986).

Feeding

Over the years there has been considerable effort placed on researching the physical and behavioural aspects of feeding in larval black flies, primarily with the aim of controlling black fly numbers due to their being pest species and vectors for disease (Chance 1970, Ross and Craig 1980). On a purely behavioural level, larval black flies exhibit four feeding strategies, namely collector - filterer (filter feeding), scraper (grazing), collector - gatherer (deposit feeding) and predator (Currie and Craig 1986). However, the most common method encountered, that of collector - filterer, is practised by nearly all species.

Collector-filterer

Before a black fly opens its head fans to filter feed it must twist its body around its long axis between 90° and 180°, because in a resting position the fans would face the substrate. This manoeuvre not only raises the head fans away from the substrate and out of the associated boundary layer, but also aligns the fans so that, when open, their concave surfaces face upstream (Chance 1970, Craig 1977b).

The actual act of filtration is passive, for black flies do not use their fans to create a flow of water or to actively capture particles, but instead use them only to intercept material in the water and pass it to their mouths for ingestion. However, Hart *et al.* (1991) clearly demonstrated that larvae can and do actively adjust their feeding posture according to the availability of food and water speed, moving their head fans further into faster water above the boundary layer in conditions of 'low food'. Black flies are further able to optimise their feeding under conditions of low food by reducing the number of flicks they make with their head fans per unit time (Hart 1986). While fans are adducted they are not filtering. Thus, by keeping the fans open for a greater period of time between adductions, larvae effectively increase their filtering time. Under experimental conditions flick rates have been seen to quadruple with increased feeding level within a single species (Hart and Latta 1986). Rates recorded for a single flick range from 0.15 sec for *S. vittatum* (Craig and Chance 1982) to 2 minutes for *S. fulvinotum* (Lacey and Lacey 1983).

The range in size of particles captured and ingested by black flies belies the simple structure of the head fans, and discounts simple mechanical sieving of the water as the only method of particle capture. Black fly larvae can feed on particles ranging in size from 0.091-350.0 μm, with the greatest efficiency over

a range of approximately 0.5 to 5.7 μm (Braimah 1987a). Thus, the range of particles most efficiently captured is smaller than the narrowest distance between two adjacent fan rays, which is approximately 35 μm (Ross and Craig 1980). Of the many theoretically possible methods of particle capture, the three given most attention are direct interception, inertial impaction and diffusive deposition (Braimah 1987a, Ross and Craig 1980). The relative importance of each varies according to the size of particles presented.

Scrapers

After filtering, scraping is the next most important method of feeding, but it comes second by a considerable margin. Unlike the reasonably sessile methods of filter-feeders, scrapers must continually forage over submerged substrates for algae and associated material (periphyton). Movement to new areas is carried out in typical larval black fly fashion, by looping (Crosskey 1990).

As well as the numerous facultative filter feeders that can scrape, three genera of black flies have evolved into obligate scrapers, species no longer able to filter (Currie and Craig 1986). The unique scraping method of *Crozetia spp.* employs short rake-like head fans which flick 2-3 times a second over the substrate removing the surface algae for ingestion (Currie and Craig 1986). Larvae of *Gymnopais spp.* and *Twinnia spp.* have evolved in a fashion similar to each other. They possess only the most rudimentary head fans as first instar larvae which are lost at the first moult, and then rely solely upon blade-like teeth on their hypostomata and mandibles for scraping algae from the substrate (Craig 1974).

Collector - Gatherers

The distinction between collector - gatherers and scrapers is that the former feed primarily on fine particulate organic matter (FPOM) and the latter on attached periphyton. However, it is unlikely that there are any larvae that actively distinguish between the two when feeding. The difference in feeding strategy is probably more a consequence of where a larva is feeding, which in turn may be species - specific and/or related to the prevailing conditions (Currie and Craig 1986).

There are a number of species that do live under conditions where deposit feeding is the only option. For example, *S. neavei*, which lives phoretically on crabs, is a deposit feeder when, along with its crab host, it becomes buried in mud for a considerable period of time. Most early instar black flies are also likely to be deposit feeders. Many possess only rudimentary fans, useless for filtering, while those with well formed fans are unlikely to be able to raise them high enough from the substrate to escape the boundary layer for them to be effective (Craig and Galloway 1986, Currie and Craig 1986).

Predators

Predation by black flies is only ever considered as an opportunistic behaviour and with small 'prey' items being captured inadvertently when filter feeding or browsing. However, there are examples of *Prosimulium spp*, particularly those from eutrophic water courses, capturing conspecific and heterospecific individuals up to 1/3 their size (Currie and Craig 1986).

Taxonomy

The identification of many black fly species is very difficult. This is partly due to their small size, but also the morphological similarity that many species have with each other. As well as the use of traditional taxonomic methods, black fly identification is now also performed using modern cytological techniques.

Morphotaxonomy

Classical morphotaxonomy still remains the most common method for keying out species of black flies, whether identifying larval, pupal or adult forms. Eggs are remarkably uniform in their appearance across species and have remained somewhat ignored taxonomically. The rigour with which morphological taxonomy has been carried out on the Simuliidae is astounding, with seemingly every structural character of each stage having been brought into play. However, this attention has by no means allowed for each of the more than 1600 species to be distinguished morphologically (Crosskey pers. comm. 1995).

A subclass of morphotaxonomy is morphometrics. Either key characters are derived from the relative proportion of two others, e.g., larval head capsule width versus length, or they are meristic, e.g., the number of fan rays per larval primary head fan. However, the use of such characters is being thrown into doubt as the number of examples of irregularities and extreme variations increases (Adler and Kim 1985, McCreadie and Colbo 1990).

Cytotaxonomy

Species indistinguishable by traditional morphological techniques are termed sibling species, yet are quite biologically distinct and reproductively isolated. In the absence of morphotaxonomy successfully distinguishing such species, the task of determining the validity of these species has by necessity been passed

on to less traditional methods. The most common non-morphological technique used is cytotaxonomy (Rothfels 1979, Rothfels *et al*. 1978).

Cytotaxonomy is the study of the banding patterns observed in polytene chromosomes after staining. The most common source for these chromosomes are the salivary glands of penultimate instar larvae, and the most common stain used is Feulgen. However, limited success has also been made in using chromosomes taken from the Malpighian tubules of adult flies (Rothfels 1979, Rothfels *et al*. 1978, Procunier and Post 1986).

Ostensibly, two species cannot be so similar that they both ultimately inhabit the same niche in the same environment. Often, after identifying chromosomally distinct sibling species using cytological techniques, physical or ecological differences between the new species and the original species complex are also found (Adler and Kim 1985, McCreadie and Colbo 1991, McCreadie and Colbo 1992, McCreadie and Colbo 1993).

The use of primary head fan ray number in simuliid larvae as an aid to identification

The number of fan rays constituting a primary head fan of a black fly larva has been used as an aid to larval identification and for species descriptions by numerous authors (e.g., Rubtsov 1956, Adler and Kim 1985, Currie 1986, Adler and Kim 1986, Craig *et al*. 1995). Rubtsov (1956), used head fan ray number as a character for larval identification and notes that there is minimal intraspecific variation in head fan ray number within populations and even between populations. It is the repeated observations of this minimal intraspecific variation that has led to head fan ray number being used as a key character in the way it is. Indeed, Adler and Kim (1985) rely upon this character in distinguishing *Prosimulium transbrachium* from other members of the *P.*

mixtum/fuscum complex. The mean number of primary head fan rays for *P. transbrachium* is 25, significantly different from the mean of 31 found in the other species of the complex.

Adler and Kim (1985) equated head fan ray number with seston load, such that a decrease in the filterable material within the lotic environment is reflected by increasing the efficiency of the head fans, expressed as an increase in primary head fan ray number. Their hypothesis is based on the river continuum scheme (Vannote *et al.* 1980), which predicts a decrease in the filterable material when travelling up a water way. Adler and Kim (1985) observed that *P. transbrachium* collected from lowland areas had fewer primary head fan rays than other members of the *P. mixtum* complex that they collected from upland areas.

Before using a morphological or meristic character for taxonomy it must be generally accepted that it is genetically fixed. For example: the significant variation in primary head fan ray number used by Adler and Kim (1985) to distinguish *P. transbrachium* from the *P. mixtum* complex should be independent of any environmental factors. However, they suggest primary head fan ray number is a product of the lotic environment in which the larvae were collected. If true, then one would not expect to find intraspecific variation in primary head fan ray number in their research, as in their paper they indicate each species studied was only collected from a single type of habitat. However, they still assume head fan ray number is genetically fixed even though larvae of each species were not collected from a range of habitats.

The only reliable method of determining if the number of primary head fan rays for a species is fixed or plastic, is to carry out transplant experiments. By

rearing larvae under controlled conditions, head fan ray number can be quantified with respect to the environmental conditions under investigation. The data can then be analysed and a decision on whether or not head fan ray number is fixed or plastic can be made.

Even prior to my investigation of the question of whether or not head fan ray number is fixed, there have been an increasing number of anecdotal reports of intraspecific plasticity in head fan ray number. Indeed, Adler and Kim (1986) describe one of the greatest observed variations in head fan ray number for a single species. They report last instar *S. vittatum* IIIL-1 as having between 31 and 57 primary head fan rays. Interestingly, this large intraspecific variation seen in head fan ray number was among isolated populations and not within a population. Variation in head fan ray number within each population studied was very low, (P. Adler, Clemson University, pers. comm. 1994). This would favour the interpretation that head fan ray number is a plastic character and can vary according to the environmental conditions within the life history of a larva.

Each head fan ray of a simuliid larva is produced *de novo* each instar from a single cell inside the head capsule irrespective of the previous structure (Craig 1974, Fry and Craig 1995). Thus, the scope for recruitment and loss of head fan rays, and modification of the head fan with respect to the environment each instar is bounded only by the genetic limits of the species. It is unlikely that black fly larvae have a large scope for morphological adaptation of their head fans each instar, or if they do it has certainly not been documented. It is more reasonable to assume that head fan ray number for a species is constrained within limits that are in accord with typically encountered variations in the habitat for the species.

Research aims

The main objective of this work is to determine if head fan ray number is a fixed or plastic character by separately rearing cohorts of larval simuliids under three controlled feeding regimes. Also to be investigated, are the spatial, temporal, interspecific and intraspecific variations in head fan ray number among five field locations. Results of the controlled rearing experiments and field collections will be statistically analysed and then discussed in light of the current information available on larval development. From the results of the experiments a conclusion will be drawn on the question of whether or not, for some species of simuliid, head fan ray number is developmentally fixed and variations seen are a consequence of microevolution, or, whether head fan ray number is a developmentally plastic character that varies in response to environmental conditions.

Materials and methods

Collection sites

Larvae were collected from a total of five sites within Algonquin Park, Ontario: Davies Bog Outflow, Lake Sasajewun Dam, North Madawaska River, Highland Hiking Trail Creek and Costello Creek. These well established collection sites were chosen for this study as they reflected a diverse range of habitats and were readily accessible on a regular basis. The characteristics of each site are listed below.

Davies Bog Outflow (D. Bog)

The Davies Bog collection site was the overflow from a beaver dam. Only a 1.5 m wide section of the beaver dam was sampled, where the water flowed directly over the top and down the side. The depth of water was only 5 to 10 cm and water velocity varied dramatically as it fell under the force of gravity down the outside of the dam from almost still on top of the dam. Black flies were typically collected from pieces of dead grass and sticks that formed the dam. Wooden stakes were also pushed into the top of the dam onto which yellow flagging tape was tied so that the tape was in the flow of water.

Lake Sasajewun Dam Outflow (S. Dam)

Lake Sasajewun has only been in existence since the artificial damming of the North Madawaska River in the 1920's for logging purposes. Collections were taken from the turbulent water that flows over a rocky stretch from the dam to the North Madawaska River below. This short stretch varies in width from approximately 5 m where it initially pours over the dam to approximately 10 m at its end. Water velocity is highly variable, starting as a torrent where the water flows over the dam and slows considerably as the outflow widens,

shallows and becomes less inclined. The entire stretch is strewn with boulders and logs that break the surface and a number of side pools about 75 cm deep. Flagging tape was tied to dead wood that was jammed in the water flow, but larvae were collected from the rocks and sticks as well.

North Madawaska River at Highway 60 (N. Mad)

Two kilometers downstream from the Lake Sasajewun Dam the North Madawaska River approaches Highway 60, where it broadens out to approximately 15 m in width and ranges from 1 to 2 m in depth. The rate of flow varied depending upon recent rainfall, but was always quite strong. Larvae were collected from a partial beaver dam that jutted out into the flow from the east bank of the river. The debris that made up the dam provided numerous points to which yellow flagging tape could be attached.

Costello Creek (Cst. Cr.)

Costello Creek was the only site not within walking distance from the laboratory. However, due to a colleague's requirement to visit the area, regular collections could still be made. The site was directly beyond a lake outflow. The outflow varied in the 20 m section sampled, beginning as a narrow stream (2 m wide, 1 meter deep) before broadening out (5 m wide, 0.5 m deep) and then being directed through a narrow culvert passing under the road (2 m wide, 0.5 m deep). The water flowed quite slowly throughout the water course until the culvert. Larvae were collected from stones that formed the bed of the creek and particularly from the abundant cattails (*Typha latifolia*).

Highland Hiking Trail Creek (HHT Cr.)

The Highland Hiking Trail Creek was the smallest water course from which larvae were collected and adequate samples were not always available. The site was downstream from a small bog formed at the outflow of a beaver dam. Strips of yellow flagging tape were secured to sticks in the narrow stream, barely 1 m across, but they often remained uncolonised by larvae.

Collection Methods

Collection of larvae for tracking head fan ray number in wild populations

Larvae were collected weekly from the five locations within Algonquin Park. A collection was simply a random sample of approximately 500 larvae of all species and age classes at the site, when availability permitted. Larvae and the substrate to which they were attached were placed in polyethylene Ziploc® freezer bags half filled with water from the collection site and were transported to the lab on ice in an insulated container. Larvae that were to be preserved for subsequent analysis were transferred into 20 ml glass scintillation vials which were then filled with Carnoy's solution (3:1, 95% ethanol : glacial acetic acid). A piece of paper, on which the date and collection site were recorded, was also placed into the vial before it was refrigerated.

Rearing apparatus

Forty individual rearing containers were constructed in which to raise black fly larvae, from either eggs or early instar to last instar larvae. Due to the number of containers required and the length of time they were to be operational, a cheap, compact and reliable design was necessary. The best aspects of two designs by Craig (1977a) and Philipson (1953), were combined to produce a suitable container in which to rear the larvae, (Fig. 3).

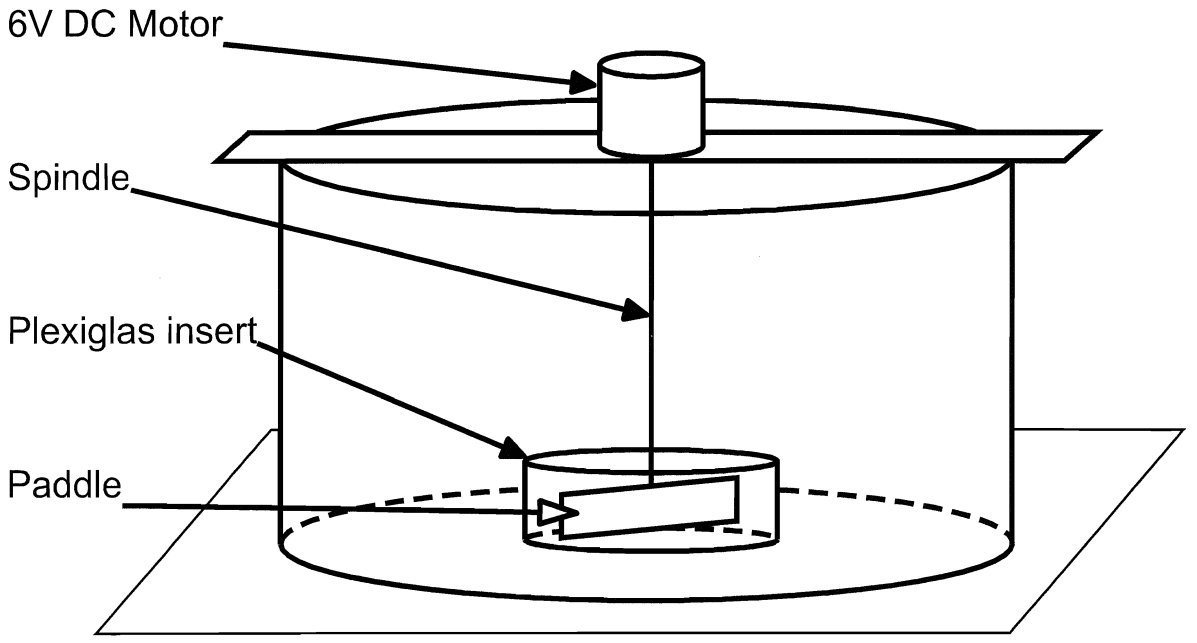

Figure 3. Apparatus for carrying out controlled experiments on Simuliid larvae, designed after Philipson (1953) and Craig (1977).

The final design for the rearing container, (Fig. 3), took advantage of a 3.1 l cylindrical frozen food container (Frig-O-Seal ®) 140 mm deep and 170 mm in diameter. A stainless steel plate 180 mm long and 55 mm wide was placed across the top of the container to support a 6V DC motor (Herbach & Rademan Co., Cat. No. TM89MTR5212). The motor, in turn, bore on a 133 mm long spindle a rectangular paddle 14 mm high and 55 mm wide with which to 'stir' the water, thus providing a current. A 26 mm high open-ended Plexiglas cylinder with an internal diameter of 63 mm was glued to the center of the bottom of the container to encompass the paddle. Two spigots were inserted into the side wall of the container, one near the bottom, and the second approximately two thirds of the way up.

The Plexiglas insert performed two tasks. First, it increased the internal surface area of the container for larval attachment, but more importantly, it prevented the larvae from being drawn into the stirrer whenever the motor was started. The lower of the two spigots was attached to the water supply via 5 mm diameter Tygon® tubing, enabling the container to be filled with minimal disturbance to the larvae whenever necessary. The upper spigot was an 'overflow' placed at a height that corresponded to the rearing container containing 1.6 l of water when the motor was running.

To reduce stress on the black flies water was gently fed by gravity into the containers. A 'reservoir' of water was held in a freezer elevated approximately 20 cm above the height of the containers. The mechanism for siphoning the water from the freezer was always in place and was regulated via a series of tubing clamps. The water was distributed among the rearing containers through 3 manifolds connected in sequence between the freezer and containers. The freezer was modified to cool water to approximately 12°C. Fig. 4 shows the

Figure 4. Overview of laboratory layout in Algonquin Park, showing the modified freezer, rearing containers, power supplies and tubing for distributing water.

placement of the apparatus in the laboratory.

Collection of eggs or larvae for controlled rearings to determine effect of feeding regime on head fan ray number and head capsule length and width

Black flies for controlled rearings were collected as either eggs or very early instar larvae. To facilitate the collection of eggs advantage was taken of the preferential behaviour of females, of some species, to oviposit on yellow substrates. Strips of yellow flagging tape, 0.5 - 1.0 m long were tied at water level to solid items such that they bridged the air/water interface at each collection site.

Early instar larvae were often collected in a manner similar to the eggs, that is, they were regularly found congregated on the flagging tape in large numbers. Indeed, larvae of all stages were found on the flagging tape and if the tape was not collected the larvae would complete their development and pupate on it. If sufficient numbers were not found on the flagging tape then extra larvae could usually be collected in large enough quantities from nearby sticks, stones and vegetation in the water course. No attempt was made to identify either eggs or early instar larvae prior to rearing, partly due to a lack of suitable keys and partly because of the handling time that would have been involved in sorting out the large numbers required.

Larvae at all stages of development are large enough that they can be seen by the unaided eye. Field collected early instar larvae for rearing were transferred to the rearing containers using a Pasteur pipette. The collection was initially poured into a white tray, contrasting with the dark larvae making them easier to see, before being sucked up either individually, or in small numbers and expelled into the rearing containers. Special care was taken not to unduly stress the larvae with rapid pressure changes, and the pipette was carefully observed

to ensure that no larvae remained attached on the inside. In this manner one hundred larvae, to within two or three larvae, could be easily transferred to each of the six rearing containers that constituted a trial.

Once in the rearing containers, larvae were reared to last instar and then identified to species. A summary of the collections made for rearings can be found in Table 1.

The rearing containers were unsuitable for adding eggs to for two reasons. Firstly, the eggs needed to remain attached to the substrate upon which they were oviposited until they hatched. Secondly, each container in a trial was to be started with 100 individuals, and accurately counting out enough eggs was impractical due to their small size and the number required over the course of the experiment. To overcome this initial problem the flagging tape bearing the eggs was cut into sections and each was placed in a 500ml mason jar (Consumers Glass ®) containing well water aerated by small fish tank air pumps (Hagen Inc.®) with air stones. Under such conditions, the larvae hatched in three to four days, at which point they could be transferred to the primary rearing containers and reared in a manner similar to early instar larval collections.

A trial was comprised of three experimental feeding levels, each run in duplicate, such that a complete trial consisted of six rearing containers. The three feeding levels were arbitrarily labelled as low, medium and high and corresponded to initial concentrations of 12.50 mg/L, 31.25 mg/L and 62.50 mg/L, respectively (0.02 g, 0.05 g and 0.1 g, respectively, per 1.6 l rearing container), of finely ground Tetramin® flake fish food every two days.

Table 1. Summary of collections made for controlled rearings.

Collection Site[1]	Collection Date	Developmental stage
N. Mad	03 May 94	Larvae
D. Bog	04 May 94	Larvae
D. Bog	21 May 94	Larvae
S. Dam	21 May 94	Larvae
HHT Cr.	24 May 94	Larvae
S. Dam	03 Jun 94	Eggs
D. Bog	07 Jun 94	Eggs
D. Bog	07 Jun 94	Larvae
D. Bog	24 Jun 94	Larvae

[1] Site abbreviations as in Materials and Methods.

Feeding procedure

Twice the amount of flake food necessary for a single rearing container was weighed out using a Mettler® BB120 balance and transferred to a mortar. The flakes were then dry ground to a fine powder, judged by eye. A small amount of water was then added to the powder and the food was then ground down further into a fine, even paste. More water was added and the food was mixed into a dense suspension and transferred to a 100 ml specimen cup and water added to bring the total volume to 100 ml. The mixture was then poured repeatedly back and forth between two specimen cups to ensure a complete even mix. Once mixed the mixture was evenly distributed between two specimen cups, before the contents of each was poured into each of the two replicates of the same feeding regime.

Rearing containers were cleaned every other day at which point fresh water and food was added. A rearing container would first be isolated from the gravity water system and the motor turned off. The water inside the rearing container would then be siphoned off into a clean white plastic container using a length of plastic tubing. Any material that had settled out onto the bottom of the rearing container was deliberately siphoned off in the process. Naturally, this process left the larvae exposed to air. Any larvae that may have been inadvertently aspirated were returned to the rearing container. The container was then reconnected to the water system and refilled before the motor was turned on again. Lastly, the food mix was added to the container in the manner described above.

Larvae were reared to last instar, distinguishable by large white or black histoblasts. Often, due to asynchronous development, at this point a number of

'advanced' larvae had already pupated. For the trials begun in May, all larvae from each of the six containers comprising a trial were preserved at the same time. However, this often led to very few last instar larvae being recovered from the low feeding level. For this reason, later trials were terminated when individual containers were considered to contain the maximum number of last instar larvae that there would likely be during the trial.

Termination of trials

Termination of the trial constituted siphoning off all the water from a container and transferring all surviving larvae into a scintillation vial of Carnoy's solution. To each vial was added a tag bearing information on sample date, feeding level, trial and a unique code for indexing purposes. Larvae from trials of the same feeding level were preserved in separate vials to allow analysis of variation between replicates.

Species identification

Keying out larvae

Firstly, larvae were checked to make sure they were last instar. To meet this requirement they had to have either highly developed white or dark histoblasts. Typically, all larvae falling into this category were removed from the sample and placed in a small petri dish. A larva would then be selected and identified to species, whenever possible, using the key of Currie (1986).

Feulgen Staining

For each sample that contained *S. venustum/verecundum* complex larvae, chromosome analysis was carried out to determine exactly which species from within the complex was present (see below). Reagents for staining were prepared as outlined in Appendix A.

1) Ten last instar larvae from a sample (those with well developed white, or dark histoblasts) were selected at random. The larvae were cut open along the mid-line of their ventral side and placed into distilled water for half an hour. The effect of this hydration was to free the jelly-like secretions of the salivary glands from the cells which contain the polytene chromosomes, thus facilitating staining.
2) The larvae were then transferred to 60°C 1N HCl, for 9 to 10 min. Hydrolysis of the chromosomes separates purine bases from the sugars, effectively exposing the sugar's aldehyde group for staining.
3) The larvae are then transferred to the Feulgen stain for 1.5 - 2 h. Leuco-basic fuchsin reacts with the freed aldehyde group to give a red/purple colour, which shows up as the characteristic banding patterns used for species identification.
4) After staining, the larvae were dabbed dry and immersed in SO_2 water, which removes excess precipitated stain from the larvae.
5) Finally, the larvae were washed in tap water after SO_2 water had been poured off to remove excess SO_2 water, before being left in the refrigerator, in tap water overnight. Ions in the tap water enhance the staining process over a period of about 12 hours.

Slide preparation for determination of cytospecies

Only the chromosomes of one larva were prepared per slide. A larva would be placed on a slide and under a dissecting microscope the salivary glands were removed, easily distinguished by their elongated shape and the purple stained nuclei within. A drop of 50% glacial acetic acid was added to the salivary glands. The larva's body was removed and preserved in 70% EtOH.

The salivary glands were then gently teased apart into several pieces before squashing. Squashing the chromosomes simply entailed lowering a cover slip onto the cells, making sure no air bubbles were trapped underneath, and tapping it down gently with a blunt instrument. The squash was carried out under a dissecting microscope to monitor the degree of squashing.

The cover slip was finally irrigated with a drop of 1% aceto-orcein, which enhances the colour of the stain. Slides were checked under a Nikon® compound microscope for quality before storage at -80 °C.

Reading of the chromosomes, for accurate species identification, was carried out by Dr. F. Hunter, using the descriptions found in Rothfels *et al.* (1978).

Measurements

Preserved larvae were looked at under a Leica® 3B dissecting microscope fitted with an eyepiece graticule at 40x magnification (40 graticule units = 1 mm). Last instar larvae were pinned, ventral side down, onto a layer of Sylgard® in a petri dish that was half filled with Carnoy's. To facilitate pinning and orientation of larvae a 'V' shaped groove was cut into the Sylgard® where the larvae were pinned.

Sylgard® is a clear, inert, silicon elastomer. Bought as a liquid, Sylgard® can be poured out into a petri dish and mixed with a curing agent, at which point it will harden to a rubber-like consistency. The hardened Sylgard® is easy to sculpt with a scalpel, pin into and is impervious to the corrosive effects of Carnoy's solution.

Three measurements were taken for each larva, as follows

1) **head capsule width**, measured as the maximum width across the head capsule in line with the eyes in graticule units at 40X magnification, Fig. 2.
2) **head capsule length**, measured as the maximum distance from the back of the head capsule to the most anterior 'bulge' in graticule units at 40X magnification, Fig. 2.
3) **number of primary head fan rays**. For the first three samples analysed (larvae reared from N. Mad 3 May 94, D. Bog 4 May 94 and S. Dam 21 May 94) head fan rays on both left and right head fans were counted. However, due to insignificant variation between the number of rays of the left and right head fans of each larva (App. B), only the number of head fan rays of the right head fan were counted in the remaining samples.

All measurements, species identification and site location were recorded for each individual larva and stored on computer for later analysis.

Analysis of head fan ray number data for field collected data

GT2-Method

Using the GT2-Method (Sokal and Rohlf 1995) 95% comparison intervals were calculated for each field sample. Results of the GT2-Method lend themselves particularly well to visual analysis. When plotted those means whose intervals do not overlap are significantly different.

Analysis of head fan ray number data

Two-way analysis of variance (ANOVA)

By using two-way ANOVA, all data gathered on head fan ray number for a single species can be combined. In combining all the data, the result of any analysis is statistically more powerful in determining whether there is a significant variation in head fan ray number with respect to feeding regime.

Plots and regression

For each trial a graph was made showing head capsule width plotted against head capsule length for all individuals. Data for each of the three regimes were plotted separately, and for each a regression line of head capsule width vs head capsule length was added. These graphs are shown in App. C.

Results

Species used for tracking head fan ray number in wild populations

Field collections were made from four sites within Algonquin Park between 16 May 94 and 4 July 94 at approximately weekly intervals (Table 2). The collections were made with the aim of discovering interspecific and intraspecific differences in head fan ray number temporally and spatially. Only those species which were found regularly and in sufficient numbers for statistical analysis are recorded in the field collections, Figures. 5 - 8.

Interspecific variation in head fan ray number

There are many examples in the field collected samples of significant interspecific variations in head fan ray number. For example, in the S. Dam collection made 5 June 94, *S. rostratum* larvae have a significantly greater number of head fan rays than *S. tuberosum* ($y = 42.6 \pm 1.6$ and 32.6 ± 2.2, respectively, $p < 0.05$), in the N. Mad collection made 4 July 94 *S. decorum* larvae have significantly more head fan rays than the *S. rostratum* ($y = 62.5 \pm 2.1$ and 50.1 ± 1.7, respectively, $p < 0.05$) larvae and in the Cst. Cr. collection made 4 June 94 *S. rostratum* larvae have a significantly greater number of head fan rays than the *S. croxtoni* larvae ($y = 49.6 \pm 1.2$ and $y = 44.3 \pm 1.8$, respectively, $p < 0.05$).

Temporal intraspecific variation in head fan ray number

Data from the field collections show that mean head fan ray number for all species varies over time, though not significantly among all samples. Some of the most significant variations seen were among the *S. tuberosum* collected from S. Dam on 5 June 94 and on 24 June 94 where mean head fan ray number increases significantly from 32.6 ± 2.2 to 44.7 ± 1.7, respectively

Table 2. Summary of field collection sites and dates for tracking head fan ray number in wild populations.

Collection Site	Collection Dates		
	May	June	July
Cst. Cr.	20, 27	4, 11, 16, 22, 28	4
D. Bog	17, 21, 29	5, 11, 18, 24	1
N. Mad	17, 26	3, 11, 18, 27	4
S. Dam	17, 21, 29	5, 11, 24	1

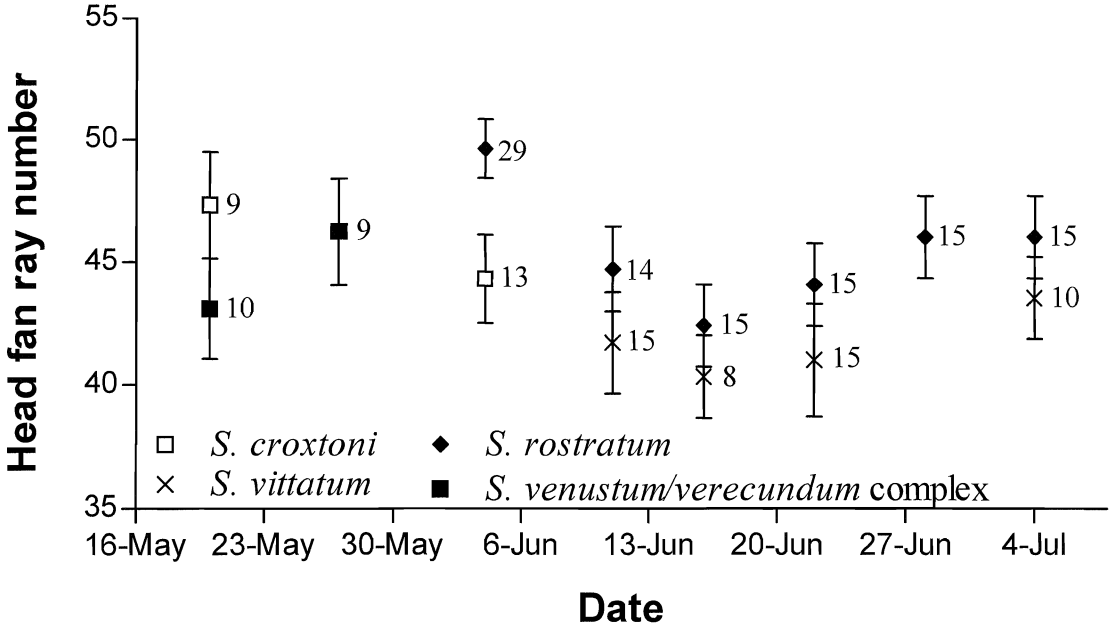

Figure 5. Mean head fan ray count, upper and lower limits of the GT-2 method, species and number of last instar larvae analysed for field collections of larvae from Costello Creek between 20th May 1994 and 4th July 1994.

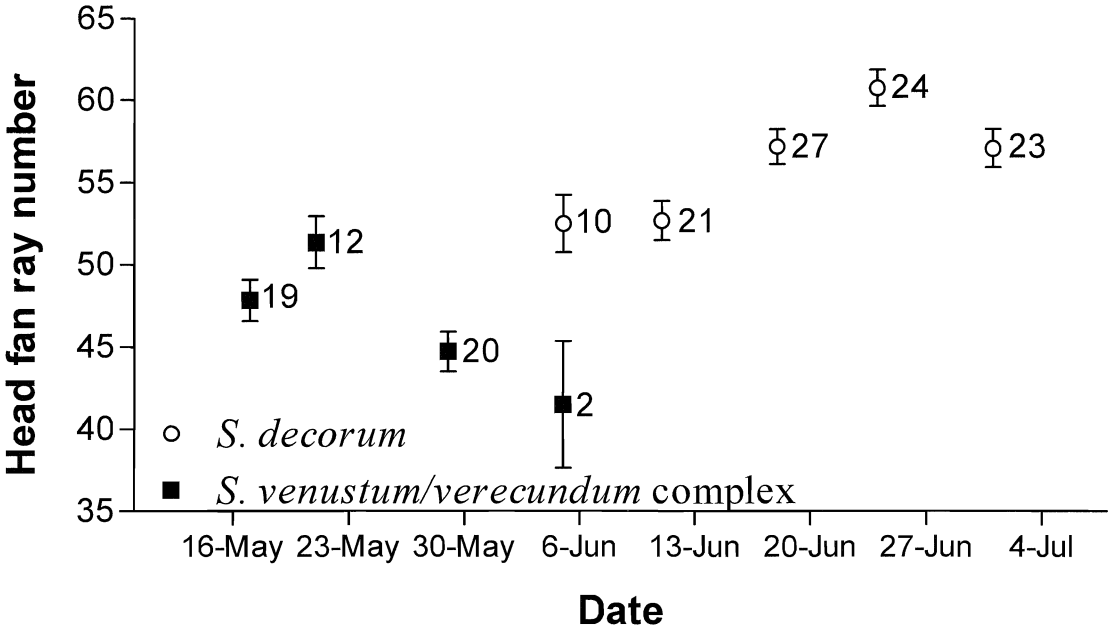

Figure 6. Mean head fan ray count, upper and lower limits of the GT-2 method, species and number of last instar larvae analysed for field collections of larvae from Davies Bog between 17th May 1994 and 1st July 1994.

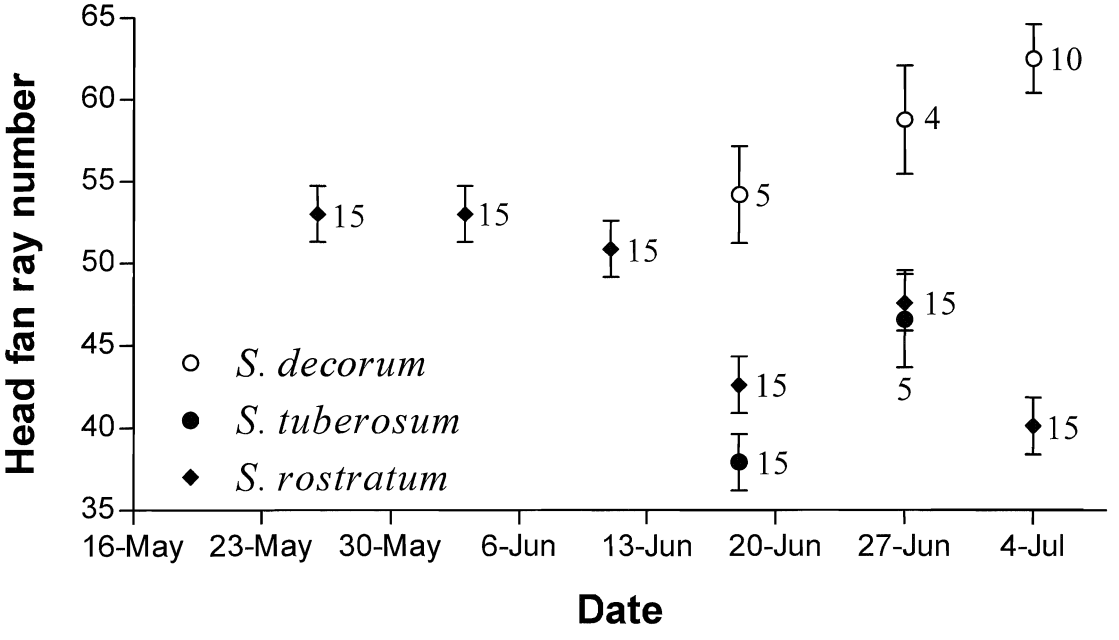

Figure 7. Mean head fan ray count, upper and lower limits of the GT-2 method, species and number of last instar larvae analysed for field collections of larvae from Madawaska River between 17th May 1994 and 4th July 1994.

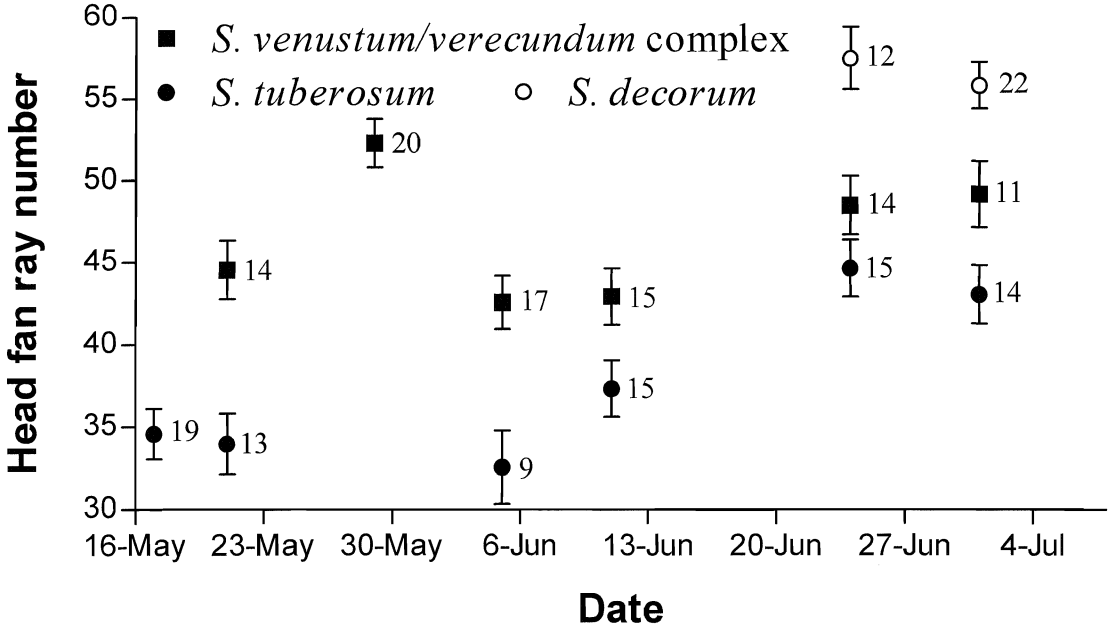

Figure 8. Mean head fan ray count, upper and lower limits of the GT-2 method, species and number of last instar larvae analysed for field collections of larvae from Lake Sasajewun Dam between 17th May 1994 and 1st July 1994.

($p < 0.05$). Further significant differences are also seen among the three samples of *S. decorum* taken from D. Bog on 11 June 94, 18 June 94 and 24 June 94, where mean head fan ray number increases from 52.7 ± 1.2 to 57.1 ± 1.1 to 60.8 ± 1.1, respectively ($p < 0.05$).

Spatial intraspecific variation in head fan ray number

Although less obvious from Figures 5 to 8, there are significant intraspecific variations in head fan ray number between two sites for samples taken close to, or on the same day. Among samples containing *S. decorum* there is a significant difference in mean head fan ray number between the sample taken from D. Bog on 1 July 94 and N. Mad on 4 July 94 ($y = 57.0 \pm 1.1$ and $y = 62.5 \pm 2.1$, respectively). Among samples containing *S. venustum/verecundum* complex there are significant differences in mean head fan ray number between samples taken from Cst. Cr. 20 May 94 and D. Bog 21 May 94 ($y = 43.1 \pm 2.1$ and $y = 51.3 \pm 1.6$, respectively), while among samples of containing *S. rostratum* there are significant differences in mean head fan ray number between samples taken from N. Mad 11.Jun.94 and Cst. Cr. 11.Jun.94 ($y = 50.9 \pm 1.1$ and $y = 44.7 \pm 1.7$, respectively).

Controlled rearings to determine effect of feeding regime on head fan ray number

Over the course of the season a total of nine rearing trials were run in duplicate from collections of either eggs or early instar larvae. The species reared, the sites from which they were collected, the dates on which they were collected and the form in which they were collected are summarised in Table 3. Trial commencement dates and termination dates for those trials where regimes were not terminated together are shown in Table 4.

Table 3. Summary of controlled feeding regimes, showing collection site, collection date, developmental stage collected and species.

Collection site	Collection date	Developmental stage	Species[1]
N. Mad	03 May 94	Larvae	*S. venustum/verecundum*
D. Bog	04 May 94	Larvae	*S. rostratum*
D. Bog	21 May 94	Larvae	*S. rostratum*
S. Dam	21 May 94	Larvae	*S. rostratum*
HHT Cr.	24 May 94	Larvae	*S. rostratum*
S. Dam	03 Jun 94	Eggs	*S. rostratum*
D. Bog	07 Jun 94	Eggs	*S. decorum*
D. Bog	07 Jun 94	Larvae	*S. decorum*
D. Bog	24 Jun 94	Larvae	*S. decorum*

[1] Members of the *S. venustum/verecundum* complex found in Algonquin Park include *S. truncatum*, *S. venustum*, *S. rostratum* and *S. verecundum*. Last instar larvae were identified to species using Rothfels *et al.* (1978).

Table 4. Table showing collection and development dates for the four trials where low, medium and high regimes were terminated on different dates.

	Trial			Regime termination	
Species	Site	Stage of development	Collection Date	Date for Med. and High	Date for Low
S. decorum	D. Bog	eggs	07 Jun 94	23 Jun 94	12 Jul 94
S. decorum	D. Bog	larvae	07 Jun 94	21 Jun 94	29 Jun 94
S. decorum	D. Bog	larvae	24 Jun 94	07 Jul 94	12 Jul 94
S. rostratum	S. Dam	eggs	03 Jun 94	21 Jun 94	03 Jul 94

Analysis of trials with respect to feeding regime

Figures 9 to 17 show the means, number of individuals and standard error for head fan ray number plotted against feeding level for each of the nine rearings.

Analysis of variance test (ANOVA)

The results of the two-way ANOVA are summarised in Tables 5 and 6 for *S. rostratum* and *S. decorum* respectively. For both species, results of the ANOVA show that there is a significant variation in head fan ray number with feeding regime, $P<0.0139$ (d.f. = 2) and $P=0.0062$ (d.f. = 2) for *S. decorum* and *S. rostratum* respectively. This variation can be seen to be due to a decrease in head fan ray number with increasing feeding regime for both species by the looking at Figures 10 to 17. The results of the ANOVA also indicate a significant inter-trial variation in head fan ray number, $P=0.0043$ (d.f. = 2) and $P<0.0000$ (d.f. =4) for *S. decorum* and *S. rostratum,* respectively.

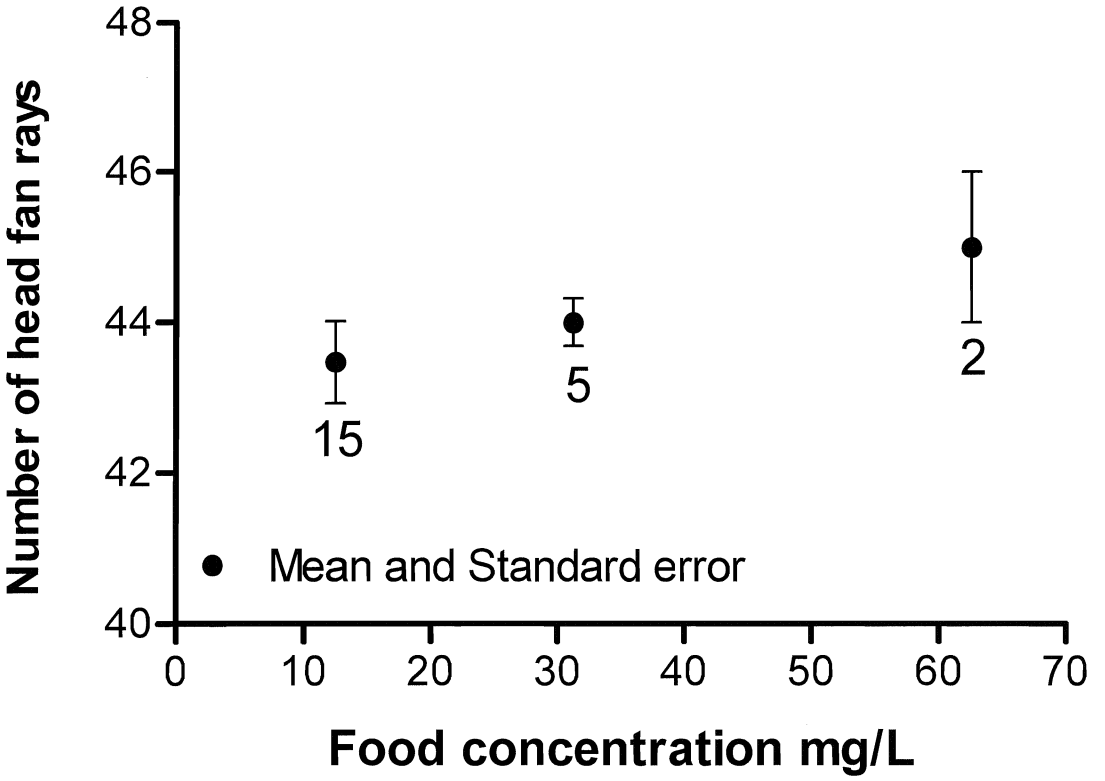

Figure 9. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. venustum/verecundum* complex collected as early instar larvae from the North Madawaska River on 3rd May 1994 and reared under each of three experimental feeding regimes.

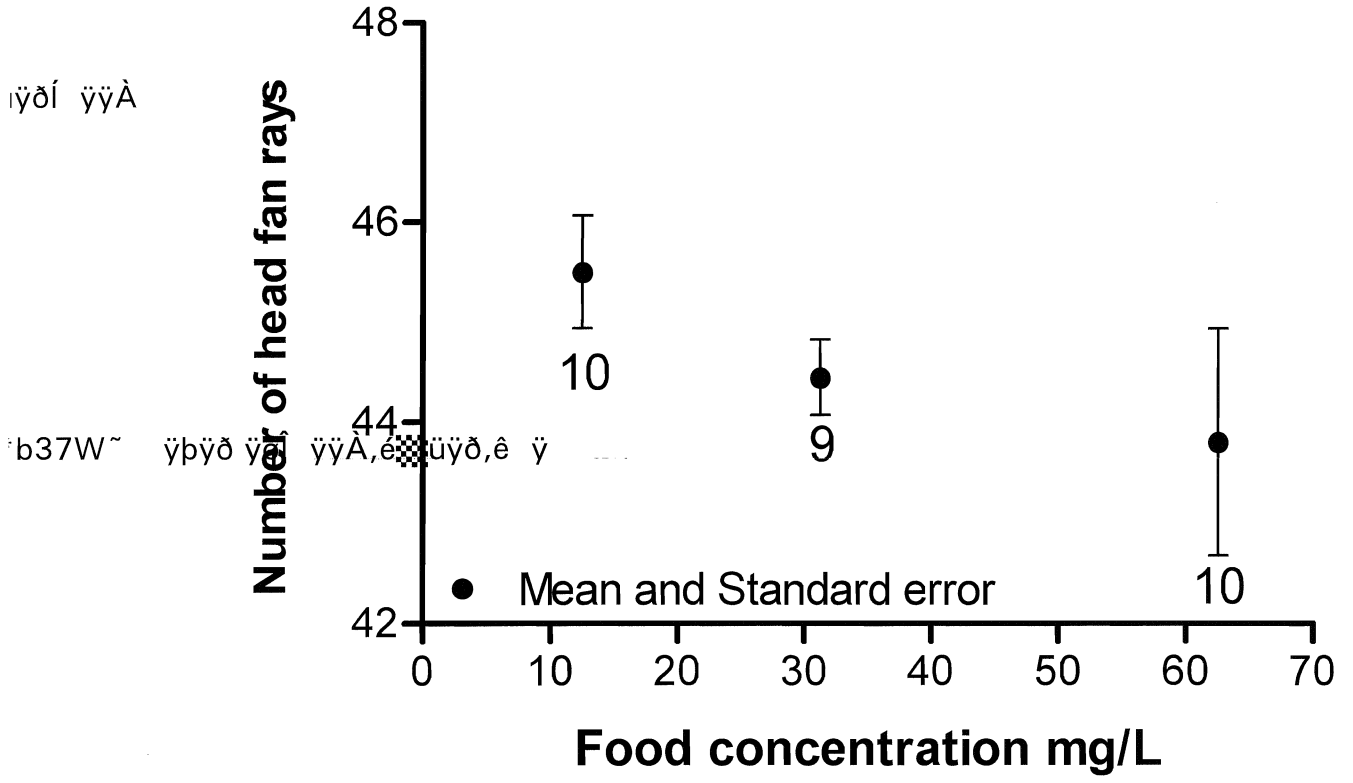

Figure 10. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. rostratum* complex collected as early instar larvae from Davies Bog on 4th May 1994 and reared under each of three experimental feeding regimes.

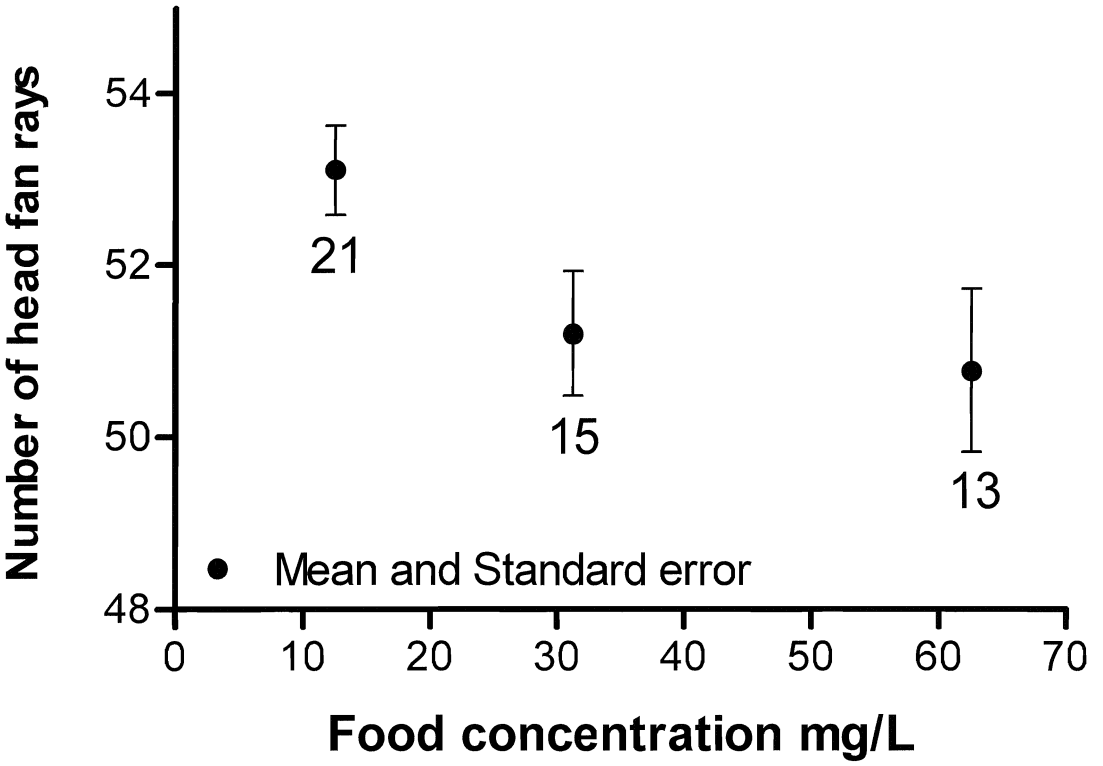

Figure 11. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. rostratum* complex collected as early instar larvae from Davies Bog on 21st May 1994 and reared under each of three experimental feeding regimes.

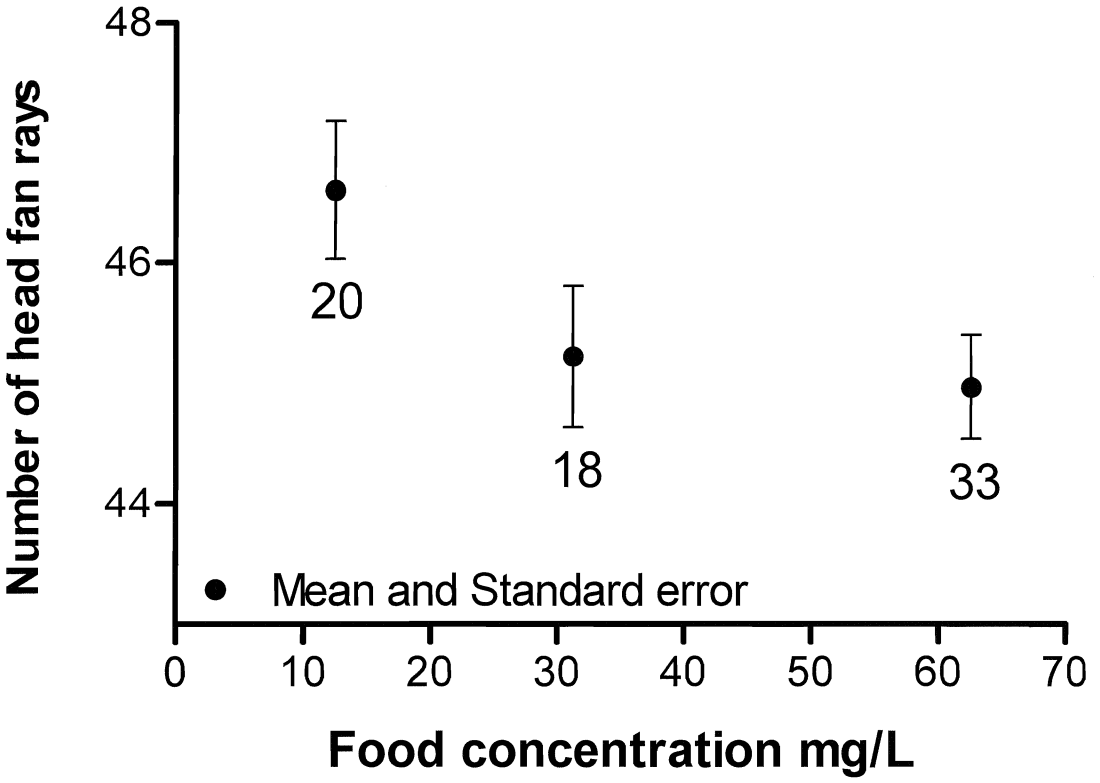

Figure 12. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. rostratum* complex collected as early instar larvae from Lake Sasajewun Dam on 21st May 1994 and reared under each of three experimental feeding regimes.

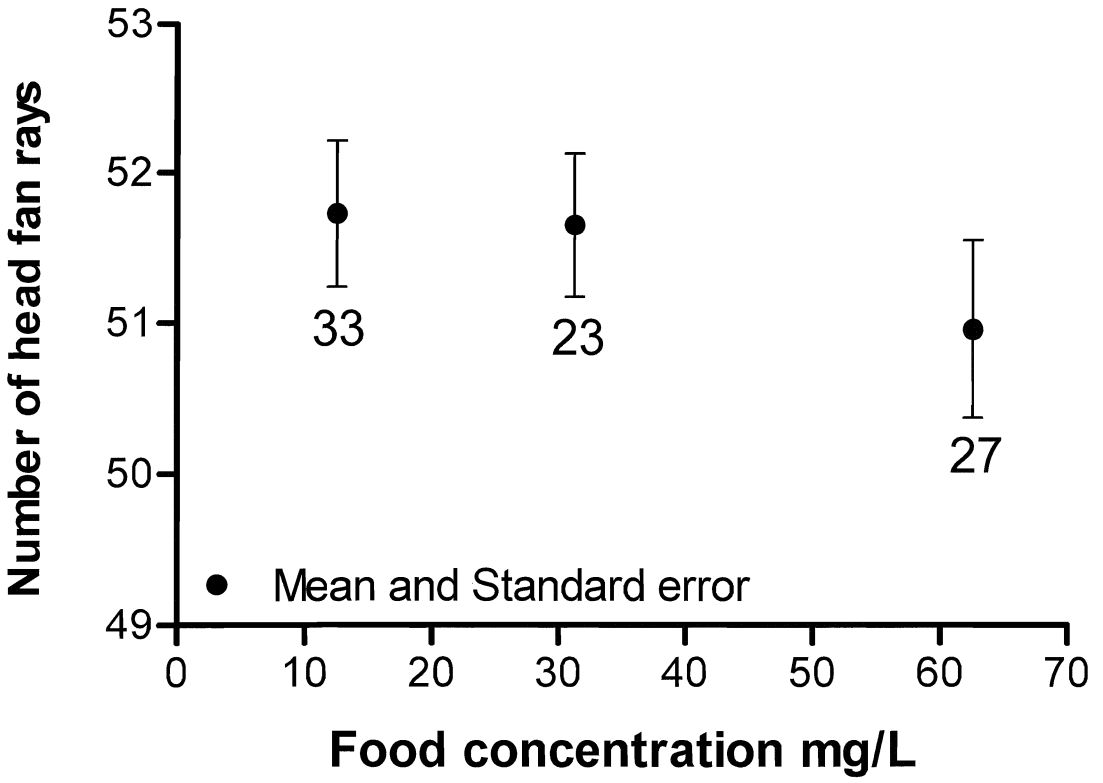

Figure 13. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. rostratum* complex collected as early instar larvae from Highland Hiking Trail on 24th May 1994 and reared under each of three experimental feeding regimes.

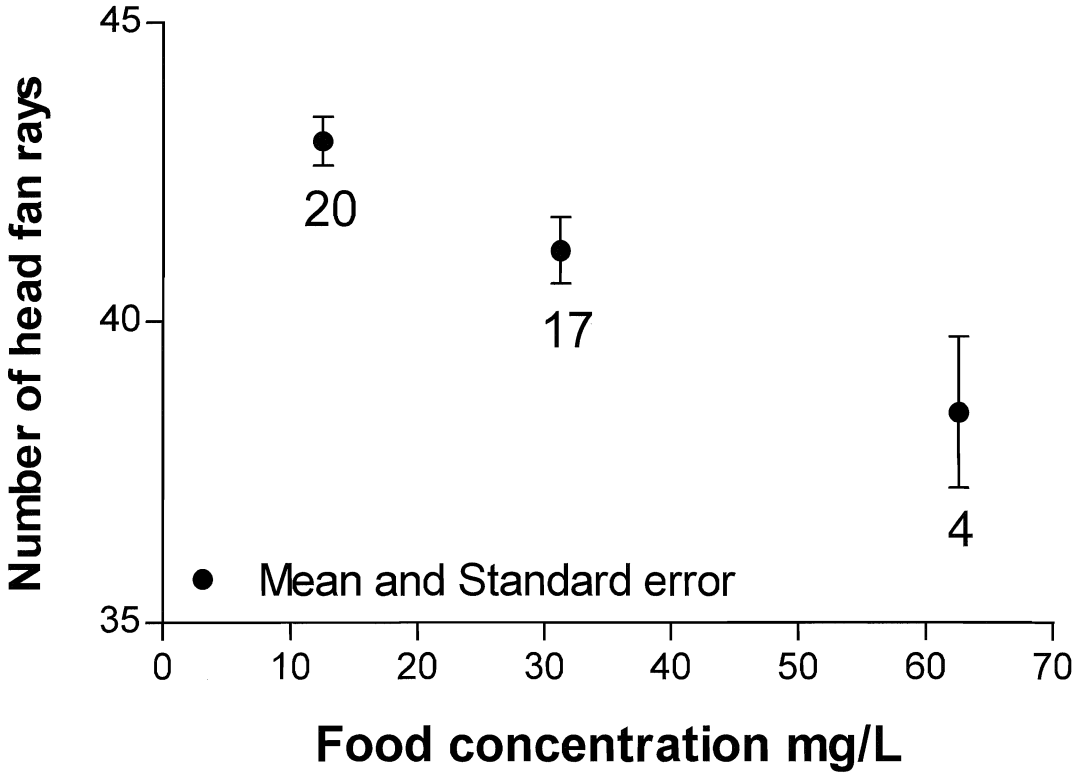

Figure 14. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. rostratum* complex collected as eggs from Lake Sasajewun Dam on 3rd June 1994 and reared under each of three experimental feeding regimes.

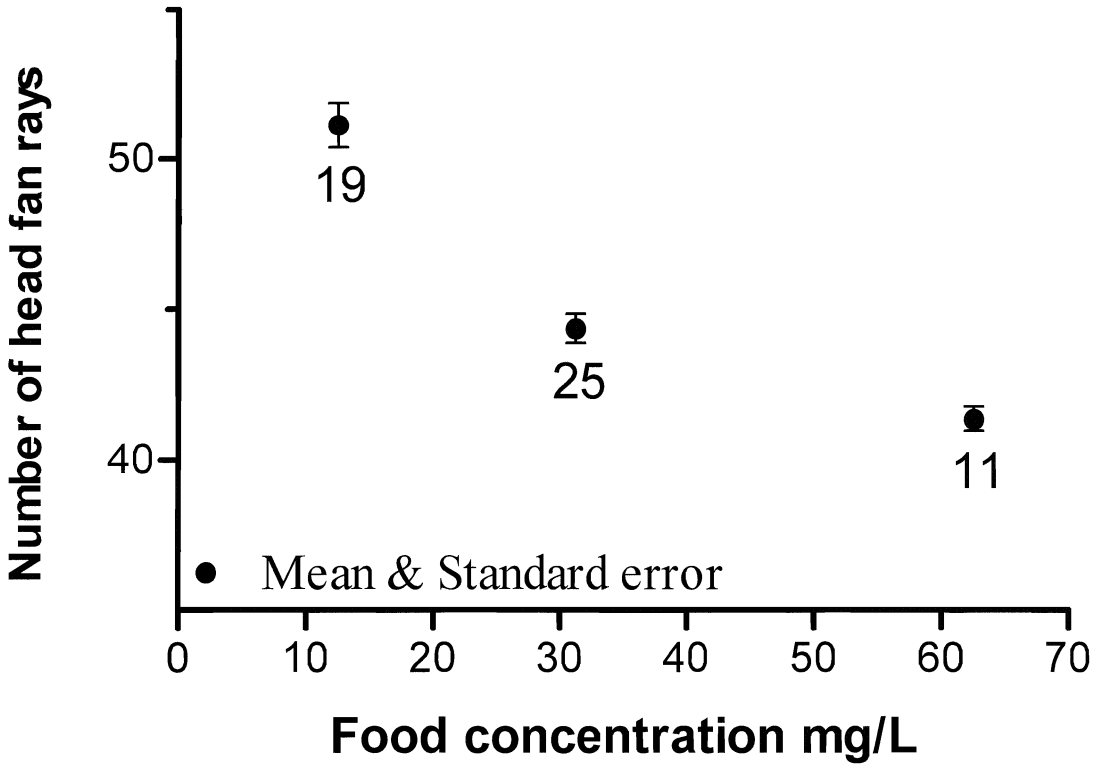

Figure 15. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. decorum* complex collected as eggs from Davies Bog on 7th June 1994 and reared under each of three experimental feeding regimes.

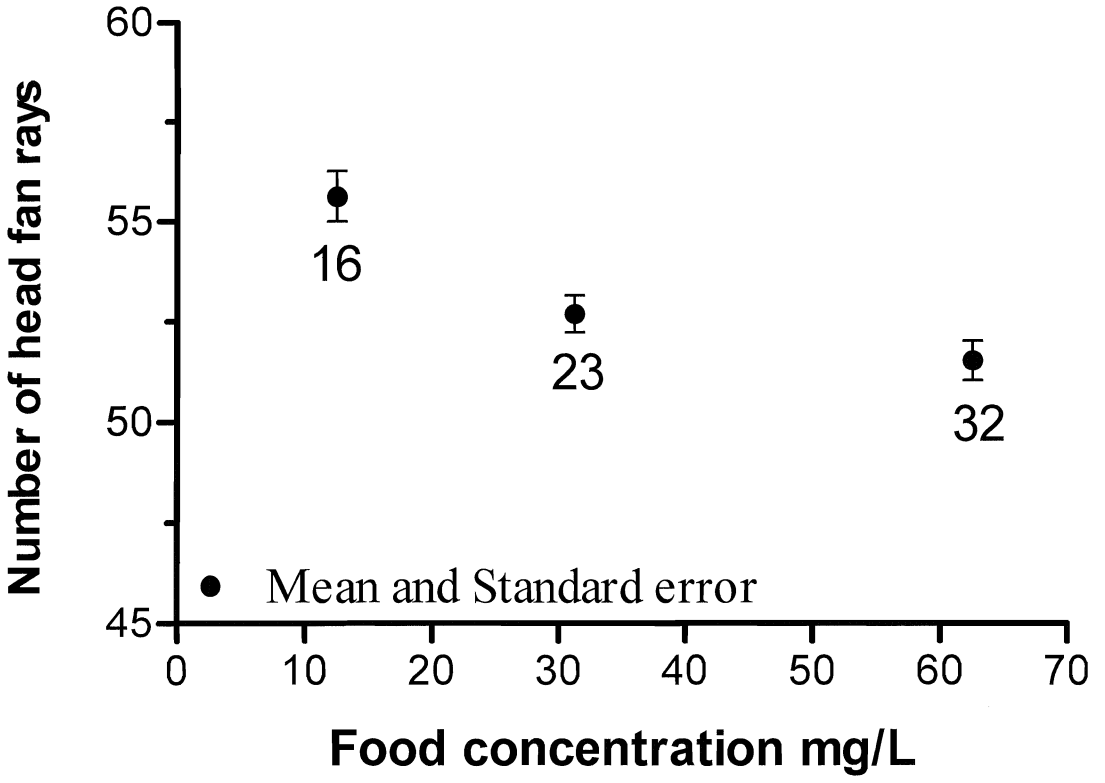

Figure 16. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. decorum* complex collected as larvae from Davies Bog on 7th June 1994 and reared under each of three experimental feeding regimes.

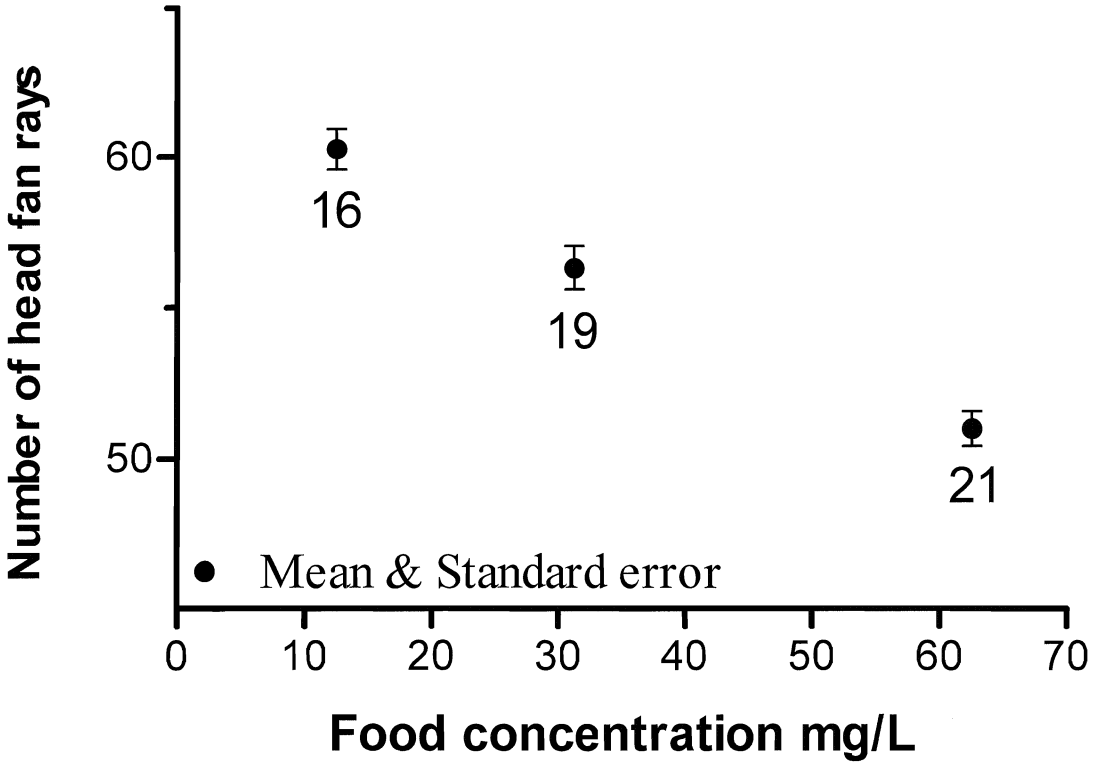

Figure 17. Mean head fan ray count, number of individuals analysed and standard error of the mean for *S. decorum* complex collected as larvae from Davies Bog on 24th June 1994 and reared under each of three experimental feeding regimes.

Table 5. Results of two-way ANOVA (F) of head fan ray number among the three feeding regimes (low, medium and high) for the five trials of S. rostratum combined. Results show sum of squares (SS), degrees of freedom (d.f.), mean square (MS), calculated F value (F), resultant probability (P-value) and critical F value (F crit.).

Source of Variation	*SS*	*d.f.*	*MS*	*F*	*P-value*	*F crit.*
Between Trials	260.2660	4	65.0665	111.4028	< 0.0000	3.8378
Between Regimes	12.0044	2	6.0022	10.2766	0.0062	4.4590
Error	4.6725	8	0.5841			
Total	276.9429	14				

Table 6. Results of two-way ANOVA (F) of head fan ray number among the three feeding regimes (low, medium and high) for the five trials of *S. decorum* combined. Results show sum of squares (SS), degrees of freedom (d.f.), mean square (MS), calculated F value (F), resultant probability (P-value) and critical F value (F crit.).

Source of Variation	*SS*	*d.f.*	*MS*	*F*	*P-value*	*F crit.*
Between Trials	170.4808	2	85.2404	28.3732	0.0043	6.9443
Between Regimes	89.7676	2	44.8838	14.9400	0.0139	6.9443
Error	12.0170	4	3.0043			
Total	272.2655	8				

Discussion

Examples of significant morphological changes in feeding apparatus in response to environmental variables are very rare. McGregor (1963) carried out three experiments, a total of forty-four individuals, investigating mouth-brush dimorphism in larval *Opifex fuscus* Hutton (Diptera: Culicidae). The mouth-brush of *Opifex fuscus* may be comprised of simple hairs or of pectinate hairs. Individuals of the two morphs and are found living together under natural conditions. McGregor (1963) found that larvae reared on a diet of fish food developed pectinate brushes, but larvae reared on an equal volume of Loeffler's dehydrated blood serum developed simple brushes. In a third experiment, larvae were fed fish food during first and second instar and then fed serum for the third and fourth instar. The larvae reared on the mixed diet all developed pectinate brushes by the second instar and by the fourth all had developed simple brushes.

McGregor (1963) hypothesised that the variation was in response to the available food. The particles constituting the serum were so small as to remain in suspension, whereas the fish food settled out. Observations on feeding behaviour showed that larvae fed the fish food predominately grazed on the bottom and sides of the containers, while those fed serum mainly filter fed. These results document a distinct dimorphic character, that of brush morphology, in relation to available food and a consequent alteration in behaviour. This is a very different system compared to the current experiment where the character, head fan ray number, is a continuous meristic variable that varies in accordance with feeding level, not food type. However, the palatal brush of culicids and the head fan rays of simuliids are likely homologous structures. Even if they are not, they develop in a remarkably similar fashion (Fry

and McIver 1990, Fry and Craig 1995).

Variation in head fan ray number

That head fan ray number is intraspecifically variable is not in doubt. Nor is there any doubt that there are significant variations in head fan ray number among populations (Adler and Kim 1986). I have shown that significant variations in head fan ray number are found not only between spatially separated locations, but also among temporally separated samples at the same site (e.g. D. Bog 29 May 94 vs. S. Dam 29 May 94 and S. Dam 11 June 94 vs. S. Dam 24 June 94). However, as has been previously mentioned, there has been no research into either the factors causing this variation or the mechanisms involved. This thesis is the first to directly investigate variability in head fan ray number and artificially influence it by controlled alteration of the environmental conditions in which larvae are reared.

For the trials of *S. decorum* and *S. rostratum* two-way ANOVA that were carried out indicate a significant variation in head fan ray number with respect to feeding regime (Tables 5 and 6). For both species this variation can be seen to be due to a decrease in head fan ray number with increasing feeding regime (Figs 10 - 17). These results appear to indicate that head fan ray number for *S. decorum* and *S. rostratum* is a plastic character that varies significantly in response to, and is inversely correlated to, food availability.

Head fan size as a developmentally plastic character

Colbo and Porter (1979, 1981) clearly demonstrated that by increasing the feeding level under which larvae were reared, development time decreased, mortality decreased, adult size increased and female fecundity increased. If we assume head fan ray number is related to feeding rate. These results would

indicate that larvae, regardless of regime should produce the maximum number of head fan rays possible to maximise fitness. Clearly, however, from these results and the findings of others (Adler and Kim 1986) this does not appear to be the case.

There is an aspect of the variation in head fan ray number observed that cannot be answered directly by the current research, and will be discussed in light of the available data and findings of other research. This is whether the difference in the number of head fan rays seen between low and high feeding regimes constitutes head fans of different area (equal ray density) or head fans with different ray densities (equal fan area). An increase in ray density would effectively decrease feeding efficiency. Due to their hydrodynamic characteristics, greater than 65% of all water directly approaching a head fan flows around it (Lacoursière and Craig 1993).

Whether or not the variations in head fan ray number seen between larvae reared under low food and high food regimes is an adaptation to the low, or to the high regime is unknown. Does the production of extra head fan rays above 'normal' by larvae in the low regime optimise feeding under poor conditions, or are the larvae in the high regime cutting back on fan ray production in response to food not being a limiting resource? At some point this question will need to be addressed by further research to determine conclusively whether the variation seen is in response to under- or over-feeding, or both.

Head fan ray number increases in response to a decrease in food availability.

If head fan ray number increases as a consequence of a reduced feeding regime we can speculate that the energetic cost of producing the extra rays is below the gain of extra food captured. Larvae from low regimes had up to 25%

more primary head fan rays than larvae from high regimes. If this translates to a 25% increase in particulate matter captured then the gains likely exceed the cost, as a 25% increase in head fan ray production could not reasonably require the 25% increase in overall food captured by a larva.

However, to assume that capture rate increases in direct proportion to fan size is grossly naive. Although actual values are not available, due to the hydrodynamic characteristics of a head fan, most of the water directly approaching them is displaced around them (Lacoursière and Craig 1993). Increasing head fan ray area by 25% is unlikely to increase capture rate to the same degree. Indeed, if head fan ray density were to increase, capture rate would almost certainly decrease.

Larval black flies already have a range of behavioural options open to them to optimise feeding. These include grazing, raising their head fans further out of the boundary layer, decreasing flick rate and selection of a new microhabitat by looping or drifting. None of these options requires the mechanisms necessary to increase head fan ray number or is likely to incur energetic costs as high as that of producing head fan rays.

Head fan ray number decreases in response to an increase in food availability

The question that needs to be answered here is: why decrease potential capture efficiency by decreasing fan size, when all experimental data suggest reduced feeding increases mortality and decreases fecundity (Colbo and Porter 1979, 1981). Even if food is not a limiting resource and a small head fan will catch as much food as a larger one, why reduce head fan ray number if the energy necessary for fan ray production is not limiting? It would seem more practical to retain a large head fan in case the prevailing conditions of plentiful

food were to change.

If the particulate load is particularly high, or made up of large and cumbersome particles, then food handling may become a problem. By decreasing head fan ray number and potentially head fan ray size, the quantity of particles captured will decrease to more manageable quantities, thus reducing handling time and optimising feeding rate. Against this hypothesis is the fact that flick frequency can be extremely rapid, e.g., 0.15 sec for *S. vittatum* (Craig and Chance 1982). It is unlikely that a fan would capture an unmanageable quantity of particulate matter in this time period.

A further hypothesis is that there is a high energetic cost to head fan production. Thus, by reducing the size of the fan produced energy resources will be freed for other forms of development. There may also be an energetic cost involved in the use of the head fans that is related to fan size. For example by reducing head fan size drag may be significantly reduced.

The above hypotheses hinge on food not being a limiting resource. If under natural conditions larvae could rely upon an unlimited food resource for extended periods, then there seems suitable scope for evolution towards exploiting this. However, the water courses in which the larvae are naturally found are unlikely to have unlimited food for any significant period of time. This puts in question whether there would be enough selection pressure on larvae, under natural conditions, for them to evolve mechanisms to exploit an unlimited food source. The alternative is, that the observed effects are a consequence of other factors.

Head fan ray variability as a consequence of the feeding regime not an adaptation to feeding regime

So far the discussion has focused on the observed variations in head fan ray number as a genetic trait, and tried to rationalise these observed trends in relation to what is known about larval behaviour and development. For head fan ray number to be developmentally plastic, larvae would require a feed-back mechanism to control head fan ray number in relation to prevailing environmental conditions. However, there remains the question of whether or not the observed variation can be explained if we assume head fan ray number is not plastic.

Number of larval instars from egg to pupation

Head fan ray number increases with successive instars (Craig 1974, Fry and Craig 1995), and larvae can pass though a variable number of instars from eggs to pupae (Ross and Merritt 1978). Although Ross and Merritt (1978) attributed variation in instar number to temperature, feeding level might reasonably have a similar effect, such that an increase in food availability and feeding rate by larvae reduces the number of larval instars. It is known that head fan ray number increases with increasing instar number, so, larvae reared in low feeding regimes may pass through a greater number of instars than larvae reared in high regimes, and consequently have a greater number of head fan rays at time of pupation.

The only conclusive way to test this hypothesis would be to carry out further experiments that look specifically at the number of instars larvae pass through in relation to feeding level. However, this hypothesis does not fully explain the observed results of the current experiment.

Ross and Merritt (1978) determined that for four species of black fly, *P. mixtum*, *P. fuscum*, *S. vittatum* and *Stegopterna mutata*, larvae developing at higher temperatures passed though six instars to pupation as opposed to seven instars for larvae developing at lower temperatures. In accordance with the above hypothesis the results indicate a trial must comprise of at least two, but more reasonably three, discrete subpopulations of larvae, each with their own number of head fan rays. This means that larvae can reach pupation at three different instars. Furthermore, a significant difference was also found between trials, indicating that pupation would have to occur over an even greater range of instars. It seems highly unlike that larvae for a species of black fly may reach pupation over a range of four or more instars, while all available evidence suggests the number of instars to pupation is highly conservative or fixed.

Another factor that is inconsistent with the above hypothesis is development time. Larvae in medium and high regimes were always terminated at the same point in time. Only larvae in low regimes were given additional time to develop to last instar (Table 4). This practice of termination would have provided additional time for larvae in low regimes to pass through extra instars over the medium and high regimes, but would not have provided time enough for larvae in the medium regime to pass through extra instars over the high regime. Yet there are still considerable differences in mean head fan ray number between medium and high regimes for three trials (*S. decorum* collected as eggs from D. Bog 7 June 94, *S. decorum* collected as larvae from D. Bog 24 June 94 and *S. rostratum* collected as eggs from S. Dam 3 June 94).

Feeding regime and differential mortality based on head fan ray number

Larval development time and mortality are related to feeding level, Colbo and Porter (1979, 1981). If feeding rate is also related to head fan ray number, with head fan ray number being random or following a normal distribution pattern spanning the limits observed in the results, the following hypothesis may explain the observed results. By a process of differential mortality, related to feeding regime, only those larvae with the optimum number of head fan rays for the prevailing feeding regime, will survive to last instar larvae. Under this scenario, only those larvae with many head fan rays will survive in the low regime, while larvae with progressively fewer numbers of head fan rays will be able to survive in the medium and high regimes.

This hypothesis provides a reasonable explanation as to why larvae from a low feeding regime would have a higher mean number of head fan rays than the population mean, but it does not satisfactorily explain the results for medium and high regimes. Larvae from medium and high regimes would be expected to have a mean head fan ray count below that of the low regime, as they do. With food less of a limiting resource, larvae with fewer head fan rays would be able to feed equally well and develop as fast as larvae with a greater number of head fan rays.

The hypothesis does not encompass a deleterious effect for those larvae with a high number of head fan rays in the high regimes. Without such an effect we would expect to see a wide distribution in head fan ray numbers in high regimes, as all larvae, irrespective of their head fan ray number, would be able to feed adequately. However, this is not the case. Standard deviations, when calculated for regimes of all trials, showed very little variation from one another.

Field collections

Interspecific variation in head fan ray number

As noted in the Results, among the field collected samples there are many instances of interspecific variation in head fan ray number, such that on the same date different species have different numbers of head fan rays (Figs. 5 - 8). Typically, when two species are found together at the same site, one of these usually has consistently more head fan rays than the other on each date that the two were collected together. This does not appear to be the case for the two samples of *S. croxtoni* and *S. venustum/verecundum* complex from Cst. Cr. taken 20 May 94 and 4 June 94 (Fig. 5). However, in Algonquin Park there are four species in the *S. venustum/verecundum* complex, and the 20 May 94 sample likely contains a greater proportion of *S. venustum* and *S. truncatum* whereas the 4 June 94 sample likely contain more *S. rostratum* (Hunter 1990). Thus, the generalisation that the relative number of head fan rays is fixed among species from the same sample may be valid for all samples.

Temporal and spatial intraspecific variation in head fan ray number

For *S. rostratum*, *S. decorum*, *S. croxtoni* and *S. tuberosum* the number of head fan rays changed significantly over the season even at the same collection site. There was only one species, *S. vittatum*, for which this site-specific temporal variation was not seen in the current study (Cst. Cr., Fig. 5). However, preliminary data collected prior to the 1994 field season (App. D.1), and research by Adler and Kim (1986), do indicate that there are intraspecific temporal and spatial variations among populations of *S. vittatum*. In addition to *S. vittatum*, larvae of *S. decorum* were also surveyed in the preliminary research (App. D.1), where temporal and spatial differences in head fan ray number were again found.

Overview of intraspecific variations

The field collections of larvae show significant spatial and temporal variation in head fan ray number. However, when graphed there is no indication that the data (head fan ray number) are correlated to either site or time of year. This leaves the question open of determining exactly what is influencing head fan ray number.

Temperature and food availability have been found to influence larval growth and development significantly (Ross and Merritt 1978, Colbo and Porter 1979, Colbo and Porter 1981, Merritt *et al.* 1982, Shipp and Procunier 1986, McCreadie and Colbo 1991, McCreadie and Colbo 1993). What remains to be determined, is the relative importance of each.

Rearing system

The rearing system was designed for acceptable functionality at the cheapest cost and this combination brought with it a number of problems. Some of these problems were factored out by experimental design while others have been taken into consideration when discussing the results.

Flow rate

During the experiments water velocity was never determined within the rearing containers, although provision had originally been made to do so. The containers, although not complex geometrically, did contain the Plexiglas cylinder insert to protect the larvae from the stirrer (Fig. 3). As the insert almost completely enclosed the stirrer and was only 8 mm greater in internal diameter, the flow rate on its inner surface would have closely approximated the velocity of the outermost edge of the stirrer. On the other hand, the outside surface of the insert, being directly protected from the stirrer, would be subject to far

slower velocities.

During the experiments black flies were found on every surface throughout the containers, even on the stirrer itself. Thus, to pick a single point in the container from which to take measurements would not represent the flow encountered by all larvae. Determining flow rate for some surfaces in the container, such as the stirrer, would not have been practical. Water velocity was instead judged by eye to approximate that of the environment from which the larvae were taken. This velocity was used throughout the experiment.

Larval black flies are quite mobile (Eymann 1991; Lacoursière 1992) and I assumed that within the limits of the range of water velocities found in the rearing containers they picked the optimum they could find (Horne *et al.* 1992). When given a range of velocities, some species choose a narrow optimum range (Horne *et al.* 1992), but still developed normally at velocities well outside this range. Eymann (1993), in field observations, found *S. decorum* and *S. venustum* living at velocities of 20 - 130 cms^{-1} and 30 - 90 cms^{-1} respectively. This range of velocities is certainly found on at least some surfaces within the rearing containers.

Water temperature

Provision was originally made in the form of a modified freezer to maintain the flow-through rearing system at a constant temperature. However, problems were encountered in maintaining a continuous flow to each of the containers in a trial. The logistical problems involved with the proposed system were solved by moving to the non-flow-through system used. Using this system, larvae were fed every other day. The method used was consistent for all feeding regimes and thus does not constitute a variable among regimes or trials.

Unfortunately, in making the system non-flow-through control over water temperature was reduced. At the beginning of the season heating within the laboratory prevented freezing, but without air-conditioning maximum temperature at the end of the season was dictated by environmental temperature. There were few particularly hot days, and the maximum water temperature recorded during the season was 25°C. Within a trial, the temperature did not constitute a variable since the containers were located directly adjacent to one another and were equal in temperature. However, among trials temperature was not consistent. Thus, only head fan ray analysis with respect to feeding level within a trial is statistically valid. Any inter-trial comparisons must be done with great care.

The effect of temperature on life history and mortality of black fly larvae is species dependent (Ross and Merritt 1978; Colbo and Porter 1981; Davis *et al.* 1992). Ross and Merritt (1978) found mortality too great at temperatures over 8.5°C to carry out statistical analysis on larvae of *Prosimulium mixtum/fuscum*. In comparison, Davis *et al.* (1992) observed that larvae of *S. yahense* developed successfully in the field at water temperatures of 25-26°C, while Colbo and Porter (1981) achieved 78-81% survival when rearing first instar larvae of *S. verecundum* and *S. vittatum* under three controlled temperature regimes of 15°C, 20°C and 25°C.

During the course of the experiment, mortality remained at a constant level with respect to feeding regime across trials. There was no observable increased mortality coinciding with the hot days.

General experimental procedure

Cleaning

During the course of a trial the containers were regularly emptied, exposing the larvae to air. This exposure time was short (less than two minutes) and the larvae did not dry out. The few larvae accidentally siphoned off when cleaning were returned to the rearing containers apparently undamaged, where they quickly re-attached and resumed filter-feeding.

The water used in all experiments was drawn from a well. Although not analysed, it was assumed to contain little or no material utilisable by the black fly larvae as a source of nutrients. Well water is typically free of living organisms (Stickney and Kohler 1990), and prior to use, the water was left to stand in the freezer for a day. Over the course of the experiment no appreciable amounts of matter settled out of the water. However, as the water used was the same throughout a single trial, before the addition of the food supplement, any errors caused by material in the well water are equal across regimes.

Observations in relation to feeding regime

The feeding levels used were chosen after experiments by Colbo and Porter (1979, 1981) to span a range of feeding regimes that would influence black fly development the greatest and cause minimal mortality. However, unlike the experiments by Colbo and Porter (1979, 1981) where feeding rate was determined on a per larva basis, it was felt more appropriate in these experiments to feed at a consistent concentration per rearing regime regardless of survivorship over time.

As found by Colbo and Porter (1979, 1981) a low feeding regime was found to retard larval development and decrease synchrony of moulting (Table 4). Due to variation in development rate it was necessary to terminate the low-food regimes of four trials at a later date than the medium and high-food regimes, three trials of *S. decorum* and the single trial of *S. rostratum* from eggs (Table 4). For the remaining trials in which *S. rostratum* was reared, all regimes were terminated at the same time. Variation in larval black fly development under conditions of low food is consistent with research by Colbo and Porter (1979). They found that in the laboratory asynchrony in *S. vittatum* development was greater than that of *S. verecundum* at low feeding regimes.

The observed effects of the low feeding regime on larval development are possibly a consequence of nutrient deficiency, as all other factors remained equal between regimes. Colbo and Porter (1979) studied the effects of larval feeding regime on emerging adult flies. They found adults of larvae reared at low feeding levels to be significantly smaller and less fecund than those reared at higher feeding levels. However, only for one trial, that of *S. rostratum* reared from larvae on 21 May 94, is head capsule size positively correlated to feeding regime (App. C.7). In the remaining trails of *S. rostratum* and the single trial of *S. venustum/verecundum* (App. C.4 - C.6, C.8 and C.9) head capsule size in final instar larvae did not vary with respect to feeding regime, indicating that the regimes did not significantly influence larval size, as one would expect if the larvae were suffering from nutrient deficiency in one or more regimes. In addition, for all three trials of *S. decorum* (App. C.1 - C.3) head capsule size is inversely related to feeding regime, which further indicates that the larvae are most probably not suffering from nutrient deficiency.

In contrast to the experiments of Colbo and Porter (1979), mortality was found to be very high in the high feeding regime. The high feeding regime used in this experiment was higher than the highest (50 x 10^{-2} mg / larvae / day) used by Colbo and Porter (1979). However, other researchers have reared larvae in food concentrations as high as 200 mg/L (Hart and Latta 1986). Thus, it is unlikely that over-feeding was directly responsible for mortality.

A situation encountered in all feeding regimes, but most noticeably in the high regime, was sedimentation of the food supplement. In the high regime this layer of deposited matter became quite thick between cleanings, and, at the end of a trial those larvae that did survive in the high regime were typically found on the stirrer or the top of the Plexiglas insert. Black fly larvae rely on water movement to bring them food and oxygenated water. It is likely that if the depth of deposited matter is too high then their body cuticle will not be exposed to the water for gaseous exchange and they will not be able to lift their head fans high enough out of the boundary layer for filter feeding.

A consequence of adding fish food to a closed environment is a reduction in the amount of dissolved oxygen and an increase in the level of nitrogenous compounds (Stickney and Kohler 1990). Asphyxiation due to oxygen deficiency is possible. Both a reduction in oxygen and increase in nitrogenous compounds would be further compounded by a proliferation of bacteria in the genus *Nitrosomonas*.

Although water quality in the containers was not tested directly, the presence of sedimented food, and the considerable die off in the high regimes would indicate possible toxic effects from over-feeding. Indeed, McCreadie and Colbo (1991) using the same experimental apparatus as Colbo and Porter (1981)

realised a significantly lower level of black fly larval survival. McCreadie and Colbo (1991) attribute this difference to the build up of excretory products due to cleaning containers once every three days as opposed to Colbo and Porter (1981) who cleaned the containers once a day.

A single trial was started using feeding regimes half that of the final levels decided upon. However, larval mortality in the low regime for this trial reached 100% before pupation. No trial was carried out using a higher feeding level than 0.01g per container as this level already seemed to be inducing mortality due to an excessive food concentration.

Summary

Each head fan ray is derived from a single progenitor cell. However, it is unknown whether recruitment of head fan rays with increasing instar is due to an increase in the number of progenitor cells, i.e., each progenitor cell produces one head fan ray, or, due to activation of cells already present. Either mechanism for producing head fan rays is very capable of varying the number head fan rays produced each instar by a considerable degree, as can be gauged by the considerable increases in head fan ray number in early instar larvae. All that is now required to modify head fan ray number in line with feeding regime from a 'normal' number of head fan rays, assumed to be a 'high' number, is a negative feedback system that first detects feeding level and then sends a message back to the progenitor cells to stop production of head fan rays in the presence of excess food. When food levels are low, the negative inhibition of the progenitor cells does not occur. Postulating the existence of a developmental feedback mechanism to influence head fan ray number is not unreasonable, especially in light of the knowledge that a behavioural one exists to alter the flick rate of the head fans in response to feeding level.

The results clearly show that head fan ray number for a population of simuliid larvae can be altered under controlled feeding regimes. They also indicate that there are intraspecific variations in head fan ray number among field populations both temporally and spatially. Each of the hypotheses presented, besides that which states head fan ray number is a plastic character that varies in relation to feeding regime, is unable to satisfactorily explain all the data. In forming a hypothesis to fully explain variation in head fan ray number, more variables will have to be taken into account than simply feeding regime, such as variable mortality and number of instars.

Conclusions

For this research I conclude that head fan ray number is a developmentally plastic character that varies in accordance with prevailing environmental conditions, one of which may be feeding level. In addition I suggest that head fan ray number should not be used as a character to aid in species identification without further investigation into the causes and extent of the observed variability.

Future research into the intraspecific variability of head fan ray number in larval simuliids should concentrate on those areas where 'confounding' variability entered into the current experiment. By this, what is meant, is that the effect of factors such as temperature and water velocity on head fan ray number should be investigated. In addition research also needs to be carried out to determine whether the number of instars through which larvae pass from eggs to pupae varies in response to feeding level.

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Appendix A

How to make the reagents necessary for Feulgen Staining

SO_2 water

To 200 ml of distilled water, 1 gram of potassium metabisulphate ($K_2S_2O_2$) (J. T. Baker Chemical Co.) and 10 ml of 1N HCl (made from standard concentrated volumetric solution (BDH®) according to manufacturers instructions) was added and mixed thoroughly. The solution was then transferred to a glass bottle, stoppered and refrigerated.

Leuco basic fuchsin, "Feulgen stain",

1 gram of basic fuchsin (S. B. EM Services Inc.) was added to 200 ml of distilled water, pre-heated to 80°C. The heat was removed and the solution stirred thoroughly. When the solution had cooled to 60°C, 2 grams of potassium metabisulphate was added and again the solution was mixed thoroughly. The solution was then allowed to cool to 50°C at which point 10 ml 1N HCl was added and the solution mixed thoroughly. The resulting solution was placed in a corked flask and left to stand for 24 hours until bleaching of the basic fuchsin was complete. Due to dissolved impurities, the solution was a straw or brown colour.

Finally the impurities were removed by the addition of and vigorous shaking with Norit (decolourising carbon). The Norit® was removed by rapidly filtering the solution though a course filter. The now clear Feulgen stain was then transferred to a stoppered darkened container and kept refrigerated.

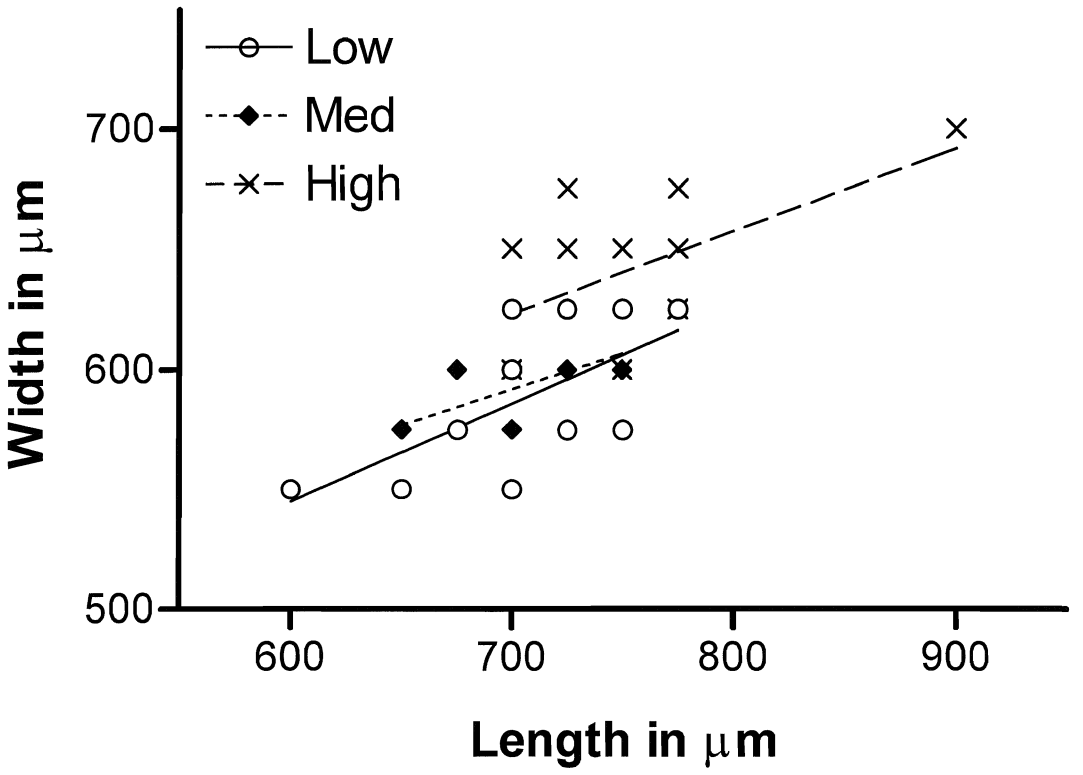
Appendix B

Results of the Students t-test (t) for intra-regime comparisons of mean left against mean right head fan ray counts, for eight counts of *S. venustum* complex, showing collection site, collection date, feeding regime, test statistic, level of probability (p) and degrees of freedom (d.f.).

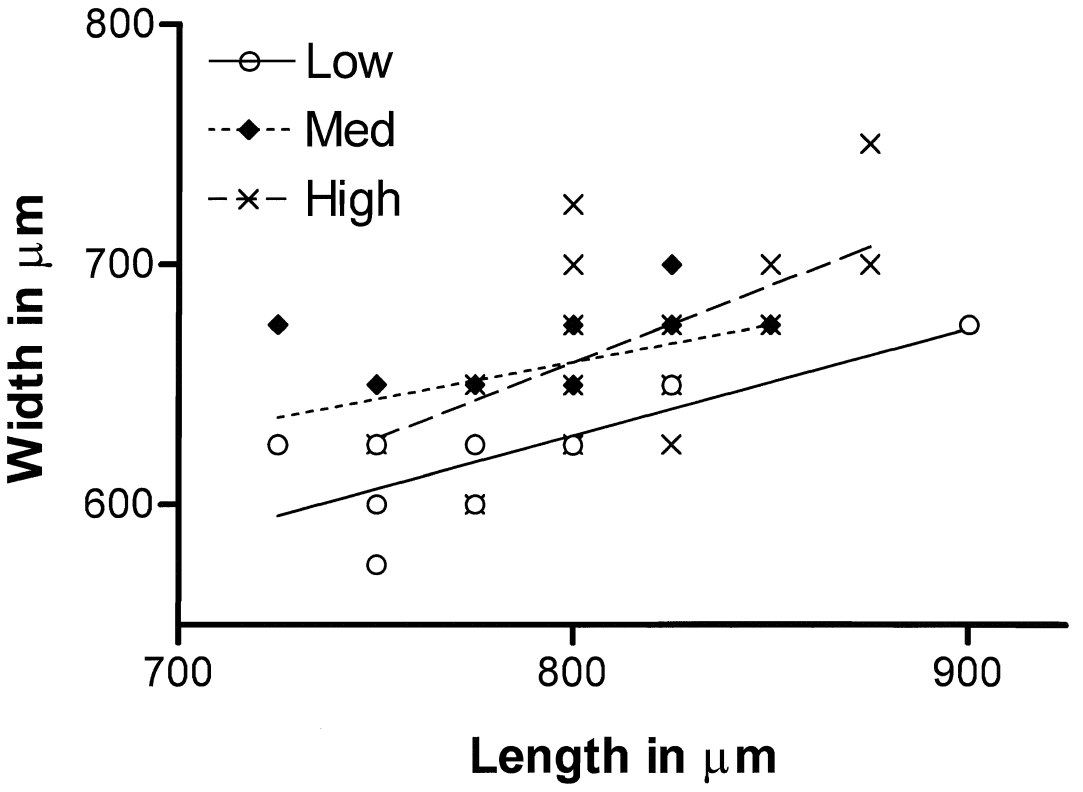
Site	Collection Date	Regime	t statistic	p = †	d.f.
N. Mad	03-May-94	Low	0.57	0.58	26
D. Bog	04-May-94	Low	0.26	0.8	18
		Medium	1.0000	0.33	16
		High	0.06	0.96	17
D. Bog	21-May-94	Low	0.07	0.95	40
S. Dam	21-May-94	Low	0.0000	1.0000	38
		Medium	0.07	0.94	34
		High	0.43	0.67	38

† $p > 0.05$ results not significant, $p < 0.05$ results significant, $p < 0.001$ results highly significant.

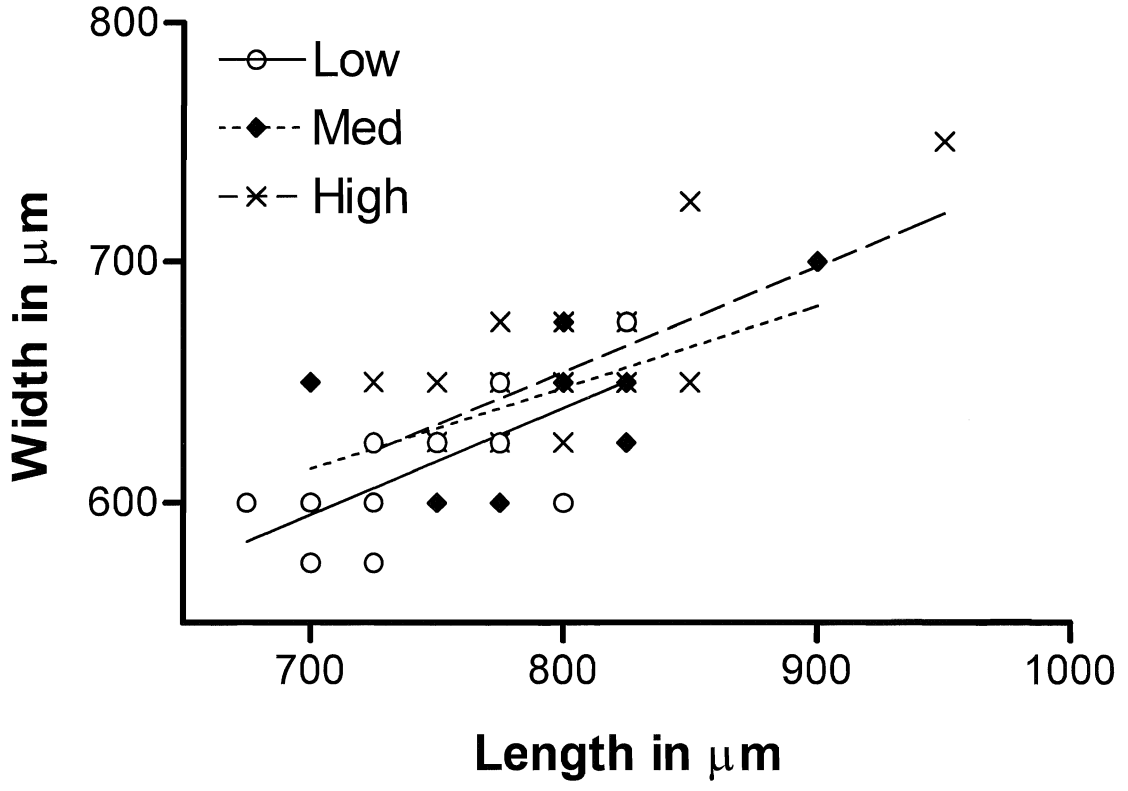
Appendix C



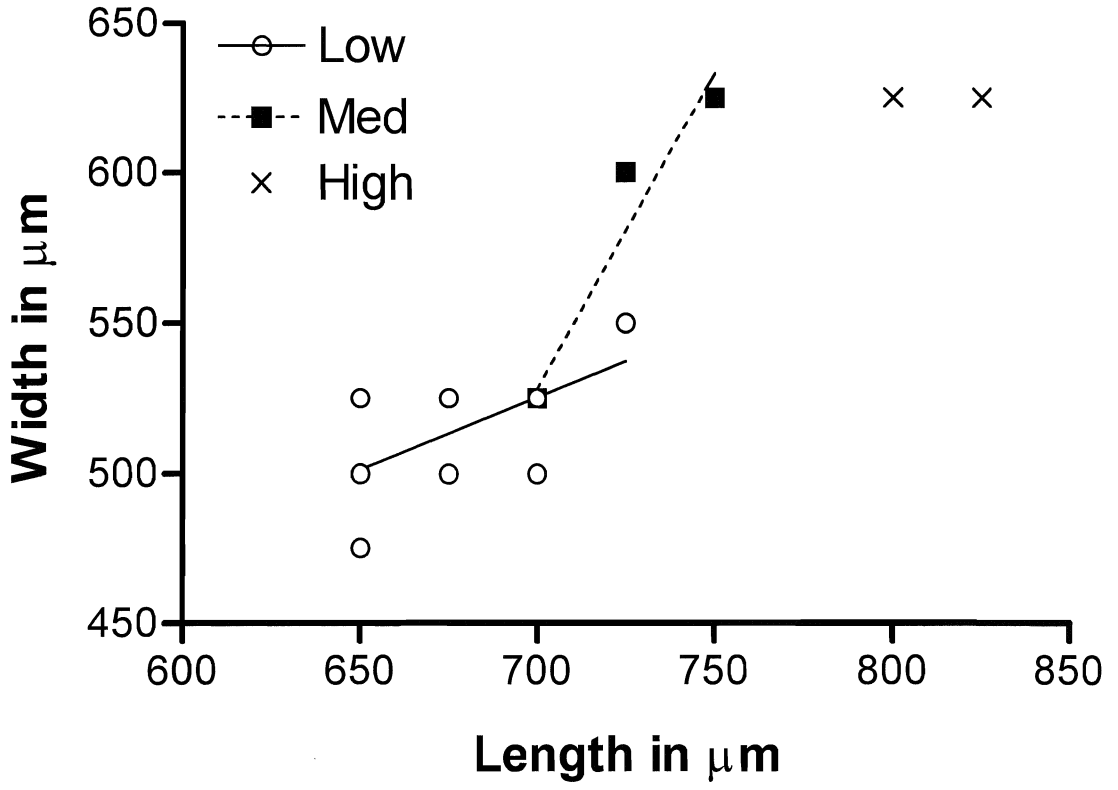
App. C.1. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. decorum* reared from eggs collected from D. Bog on 7th June 1994.



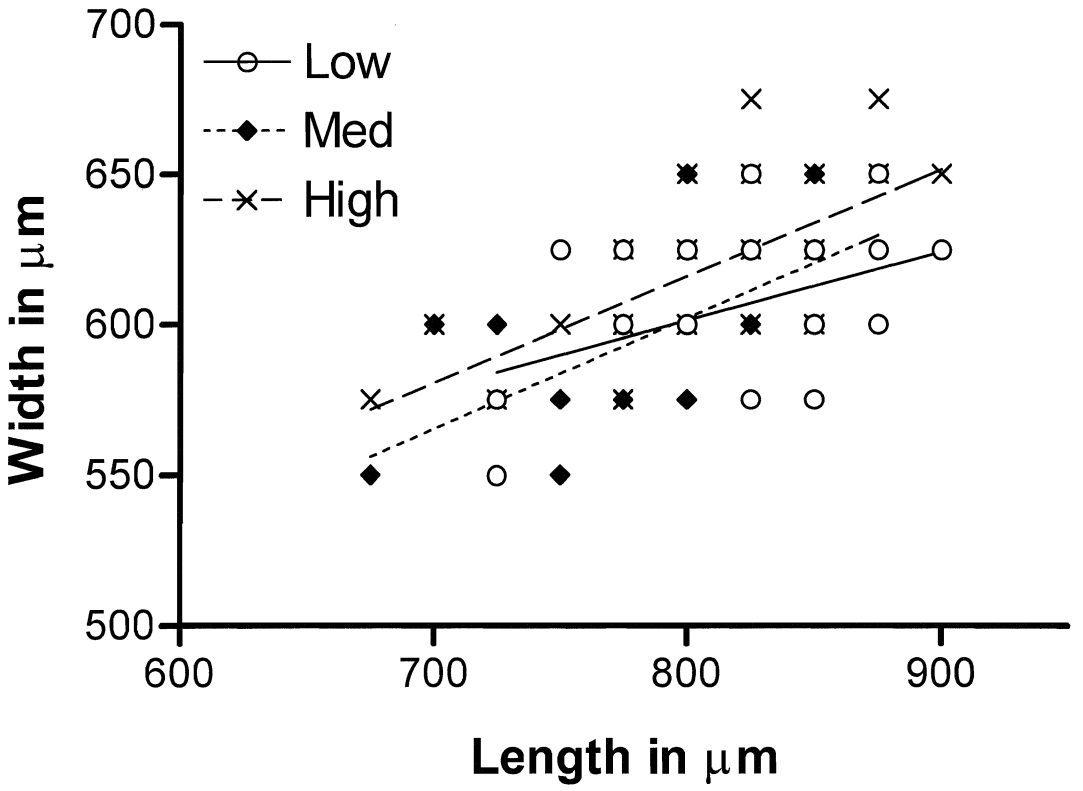
App. C.2. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. decorum reared from larvae collected from D. Bog on 7th June 1994.*



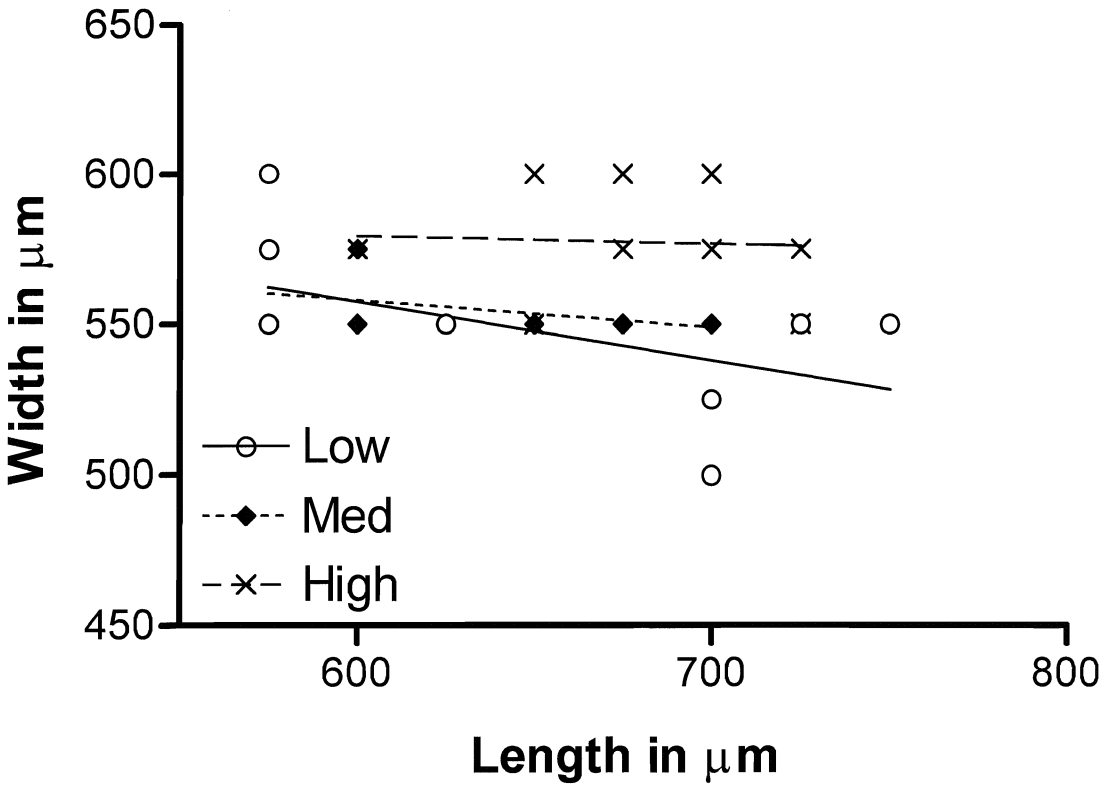
App. C.3. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. decorum reared from larvae collected from D. Bog on 24th June 1994.*



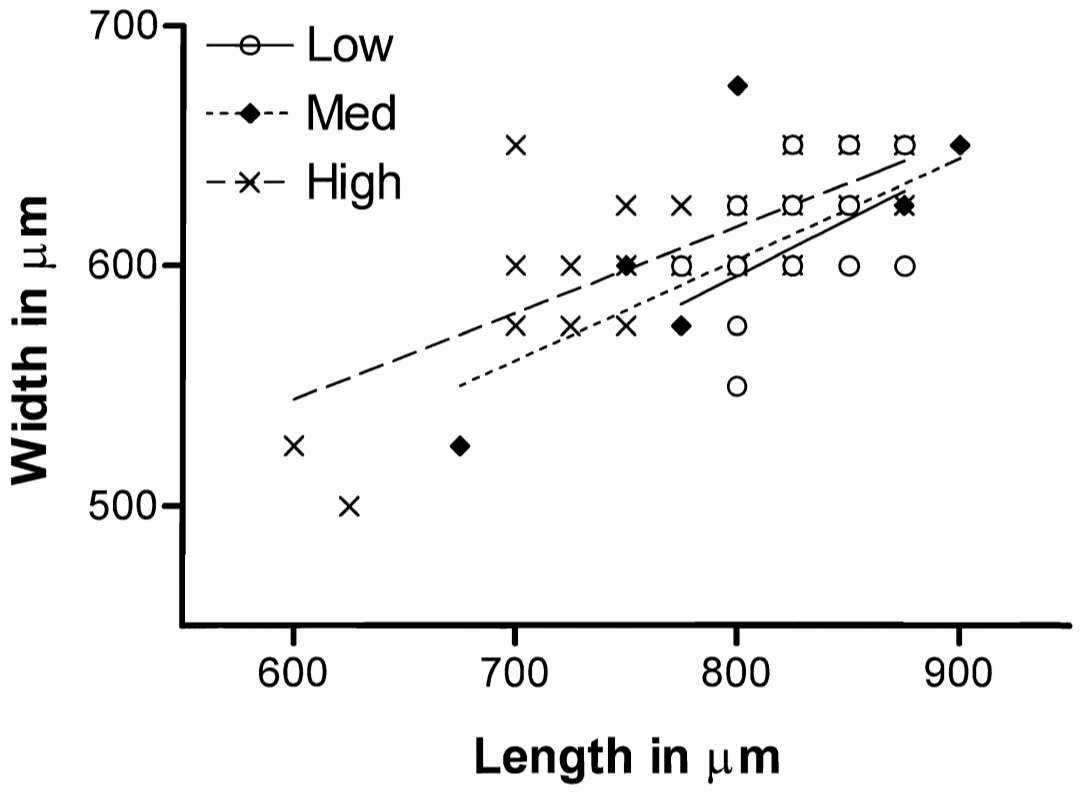
App. C.4. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. venustum/verecundum* complex reared from larvae collected from N. Mad on 3rd May 1994.



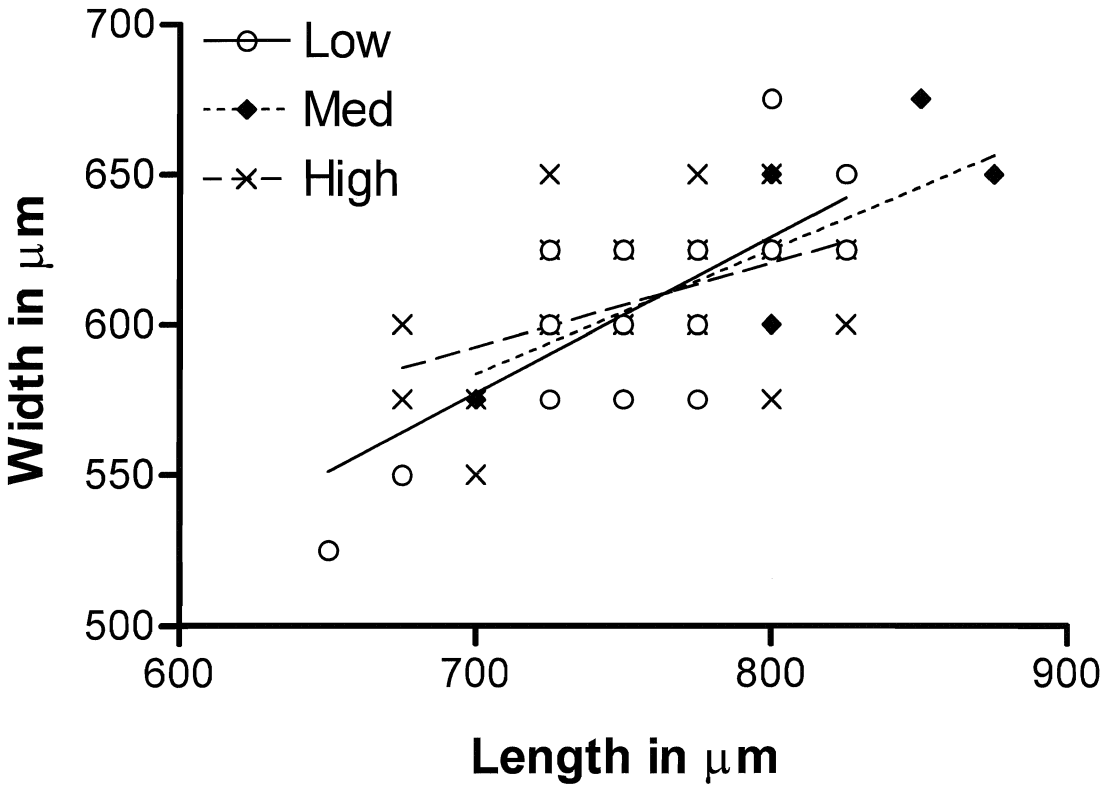
App. C.5. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. rostratum* reared from larvae collected from D. Bog on 21st May 1994.



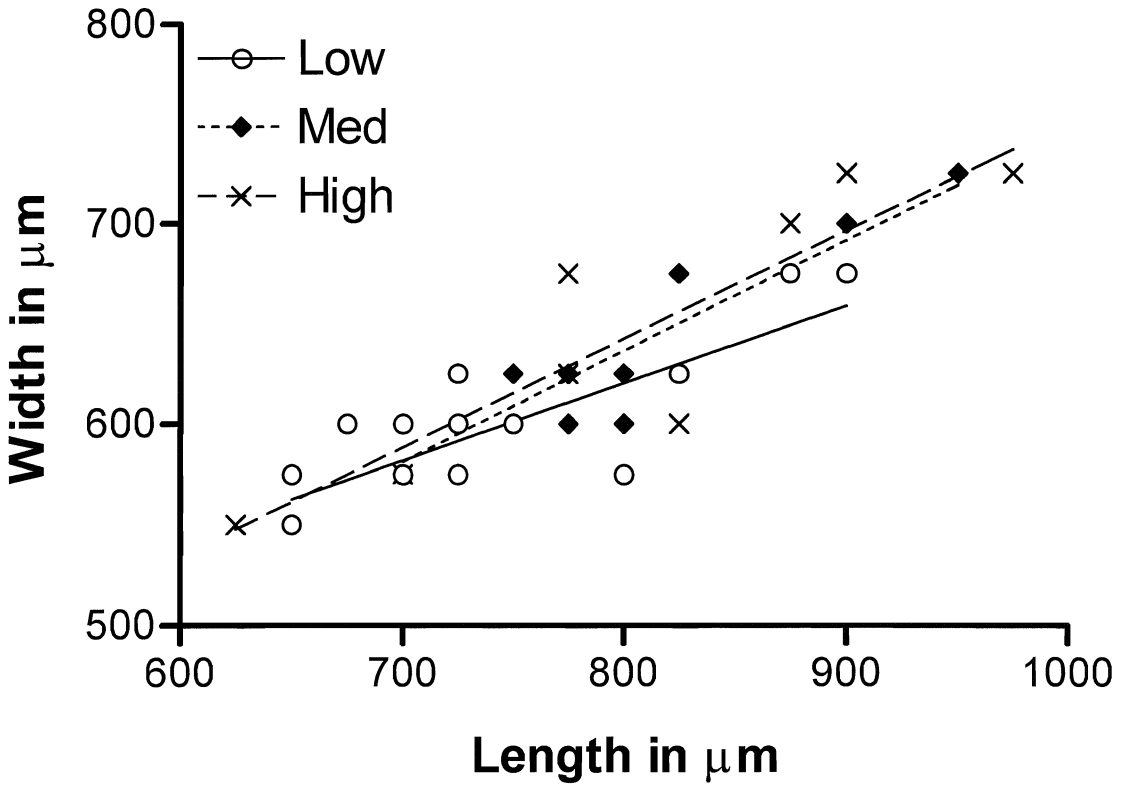
App. C.6. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. rostratum* reared from larvae collected from D. Bog on 4th May 1994.



App. C.7. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. rostratum* reared from larvae collected from S. Dam on 21st May 1994.

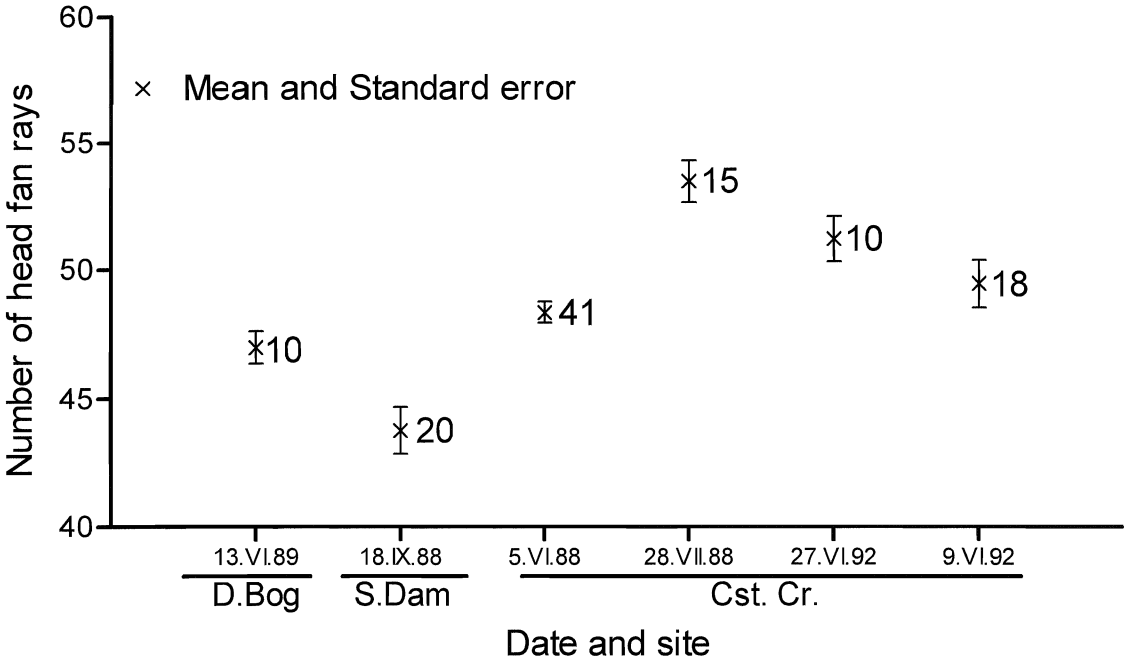


App. C.8. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. rostratum* reared from larvae collected from HHT. Cr. on 24th May 1994.

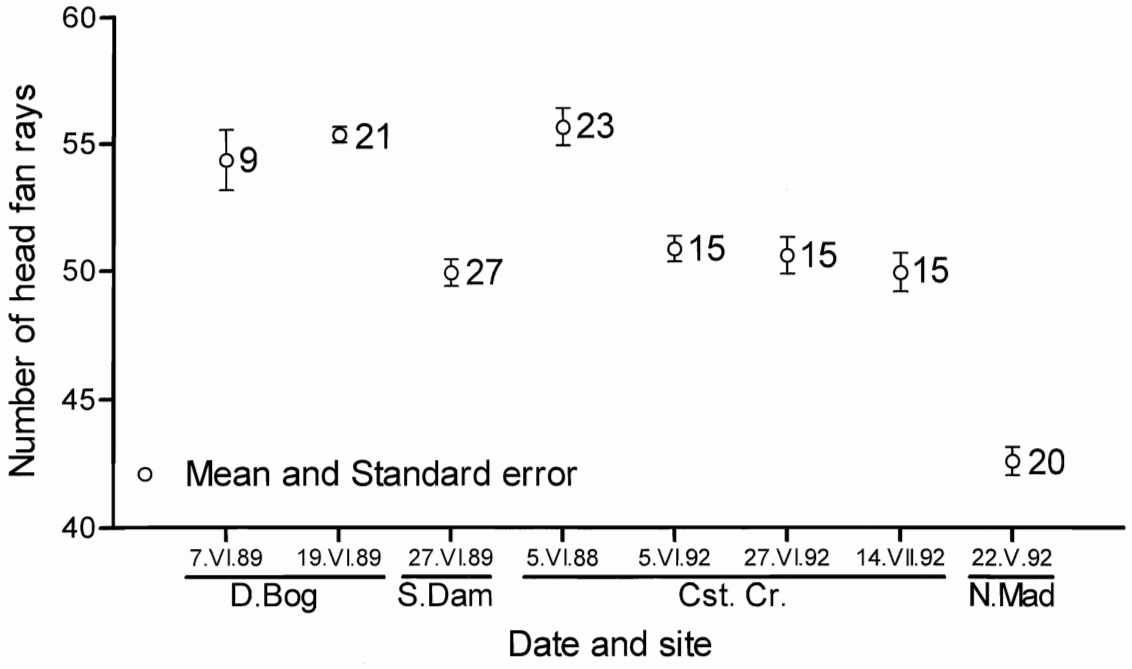


App. C.9. Head capsule width plotted against head capsule length and calculated regression line for each feeding regime of *S. rostratum* reared from larvae collected from S. Dam on 3rd June 1994.

Appendix D



App. D.1. Mean head fan ray count, standard error of the mean and the number of individuals per sample for preliminary analyses conducted on samples of *S. vittatum*.



App. D.2. Mean head fan ray count, standard error of the mean and the number of individuals per sample for preliminary analyses conducted on samples of *S. decorum*.